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VASOCONSTRICTION IN RENAL HYPERTENSION ABOLISHED BY PITHING[1]

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Although no vasoconstrictor substance has been demonstrated in the blood of hypertensive patients, or in that of animals with experimental hypertension, most investigators of the problem incline to the belief that a peripherally active vasoconstrictor substance must be the actual cause of the increased arterial resistance (Goldblatt, 1940, summary of literature). Heymans and his colleagues, impressed with the rôle normally played by the carotid sinus and aortic reflexes, also believe that peripheral vasoconstriction and sensitization of vasomotor endings play a part, but insist that the reflex control mechanism must also be affected in order to have sustained arterial hypertension (Heymans, 1938).

Several years ago we reported (Dock and Rytand, 1934) that rats with renal hypertension had a prompt fall of pressure to the same level as controls when both groups had the central nervous system destroyed, but this evidence was obtained with indirect and unsatisfactory methods of measuring arterial pressure. That experiment has now been repeated, using rabbits and direct recording of arterial and venous pressure and the earlier observations have been confirmed. It is known that renal hypertension may occur after the sympathetic chains have been removed (Alpert et al., 1937; Freeman and Page, 1937; Heymans et al., 1937) or the cord below C4 destroyed (Glenn and Lasher, 1938; Glenn et al., 1938). Nevertheless the nervous system does play an important rôle in the mechanism of renal hypertension since pithing abolishes the difference between hypertensive and normal animals.

METHODS. Normal rabbits or those which had been hypertensive for months due to a tie narrowing the renal artery were anesthetized, the lumbar cord exposed by laminectomy, and arterial pressure recorded continuously from a carotid artery. The cord was destroyed along with the dorsal part of the brain by pithing with a wire rod through the laminectomy opening, after tying both carotids and starting artificial respiration. Epinephrine, pitressin or renin were given intravenously through a jugular cannula and in nine animals the jugular venous pressure was observed in a manometer or recorded graphically. In two hypertensive and two control animals urethane anesthesia was used; the arterial pressure was low and the fall in relation to that in the central artery of the ear, measured indirectly before anesthesia, was much greater in the hypertensive than in normal animals. In three control and five hypertensive animals the splanchnic arterial bed was ligated before pithing. This prolonged the effect of epinephrine injections but did not prevent the fall of pressure due to pithing. The results to be reported were obtained with ether anesthesia and intact circulation, except for carotid ligation. In three hypertensive rabbits intravenous infusion of acacia-Locke's solution was used to raise venous pressure after pithing. Three hypertensive and two normal animals were pithed and the pressure allowed to fall to zero; in six hypertensives and fourteen normals repeated doses of epinephrine were given after pithing, in seven normals pithing was performed during continuous epinephrine infusion (two of these, which had many ectopic beats before pithing, went into ventricular fibrillation with immediate fall of pressure to zero on being pithed). In five normals pithing was performed at the height of a response to renin, generously supplied by Dr. I. H. Page of the Lilly Laboratories. In five hypertensive rabbits pithing was performed 30 hours after removal of the single kidney; all had normal levels of carotid pressure before being pithed.

RESULTS. In the normal rabbit there is no rise or a rise of less than 20 mm. Hg in arterial pressure during pithing. As pithing is completed there is an abrupt fall of pressure. This goes over into a more gradual fall at 30 to 50 mm., going steadily down or it may almost level off and decline gradually to zero. Prompt rise in pressure can be produced by epinephrine injection if the pressure has not fallen below 10 or 15 mm. Rabbits which have been hypertensive for months, but whose pressures fall to normal within 24 hours after total nephrectomy, react to pithing and to epinephrine in exactly the same way as normal animals.

When the arterial pressure of the normal rabbit is at a high level during continuous epinephrine infusion, there is no fall or only a transient fluctuation in arterial pressure on pithing; the pressure falls steadily to zero on discontinuing epinephrine. The effect of pithing rabbits at the height of response to renin was variable. In one, hypertension continued unchanged

for several minutes, in others there was a transient or a moderate but sustained fall, but the pressure always leveled off at a relatively high level, and declined very gradually as the effect of renin wore off. A second dose of renin, given to the pithed rabbit as the effect of the first dose wore off, again caused a rise. Thus renin, which with blood serum produces a vasoconstriction in isolated tissues of cats and dogs (Page and Helmer, 1940), has an effect somewhat like that of epinephrine. Its effect is very different from that of the humoral substance which causes hypertension of renal origin.
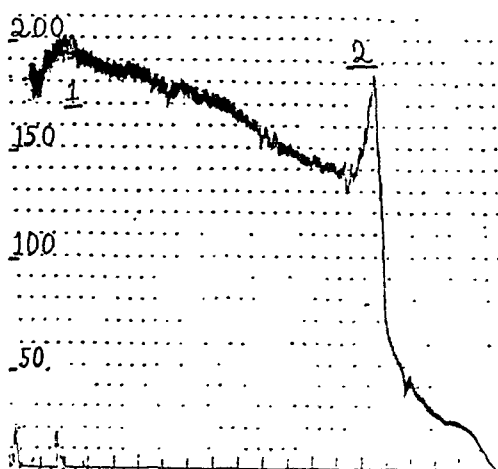
Fig. 1   Fig. 2

Fig. 1. The initial rise in pressure, in this rabbit with renal hypertension, followed the starting of artificial respiration, *1*, and a fall occurred as the ether anesthesia became more profound. At *2* the animal was pithed and the pressure rose 40 mm. before its abrupt decline and more gradual fall to zero. Time record, 20 sec. intervals.

Fig. 2. Venous pressure (upper curve, in mm. H_2O) and arterial pressure (lower curve, mm. Hg) during four responses to epinephrine (15 gamma per kgm.) in a pithed normal rabbit. No rise in venous pressure precedes the rise in arterial pressure, save for the insignificant effect of the injection into the opposite jugular. It is quite obvious that the fall of arterial pressure is due to loss of arterial tone, not to low venous pressure. Time record 20 sec. intervals.

As contrasted with normal or recently nephrectomized hypertensive animals, rabbits with renal hypertension show more marked rise in pressure on ligating the second carotid artery, on starting artificial respiration, and during the destruction of the cord (figs. 1, 3). After the cord, medulla and pons of the hypertensive rabbits are destroyed by pithing, the pressure falls abruptly, reaching 20 mm. in one or two minutes. Occasionally, as in some normals, the pressure falls sharply to 30–50 mm., and then more slowly, reaching 20 mm. in 3 to 5 minutes. If epinephrine is given there is a sharp transient rise in pressure (fig. 3). Animals may be kept alive for several hours by repeated or continuous injections of epinephrine but they

show no recovery of tone; as soon as the epinephrine is stopped the pressure falls to zero. Ligation of the coeliac axis prior to pithing (3 hypertensives, 5 controls) modifies the result very little. It does prolong the effect of a dose of epinephrine, presumably by preventing its destruction in the liver. Raising the venous pressure by infusions of 10 to 15 cc. of Locke's solution or acacia-Locke's, per kilo of rabbit per minute for three to five minutes, does not suffice to maintain arterial pressure levels over 40 mm. Hg, although at the end of infusions, with arterial pressure steadily falling, venous pressure levels are 3 to 5 cm. H$_2$O above normal. On giving epine-
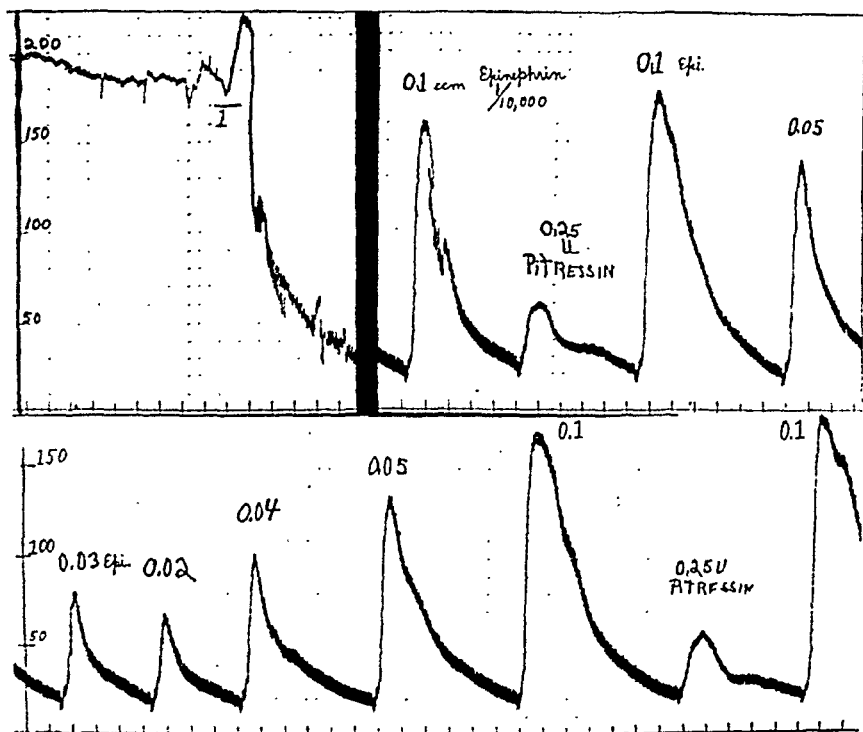
Fig. 3. Pressure in this hypertensive rabbit (3.8 kgm.) rose 35 mm. during pithing; abrupt and then gradual fall afterward. The maximal responses to epinephrine were obtained after 15 minutes. The first response to epinephrine was greater following each dose of pitressin. Time record, 20 sec. intervals.

phrine the pressure rises very sharply in the carotid, but the venous pressure either stays constant or with large doses stays at its original level for several seconds and rises after the arterial pressure has begun to fall from its peak (fig. 2). If the low arterial pressures were due only to inadequate venous return, no rise could occur following epinephrine until the venous pressure had risen markedly. Since this was not observed in any of the numerous cases in which venous pressure was noted, in hypertensive and control rabbits the fall in blood pressure must be ascribed to loss of arterial resistance rather than decrease in venous return after being pithed.

The response to epinephrine was more marked in the pithed hypertensive rabbits than in the controls or in hypertensive rabbits nephrectomized completely 30 hours before pithing. In order to minimize the effect of artificial respiration on the size of the response to epinephrine, varying doses were given repeatedly to an animal, and ventilation was increased until no further increase in response to each dose level was observed. It was always apparent that the maximal response to any given dose was
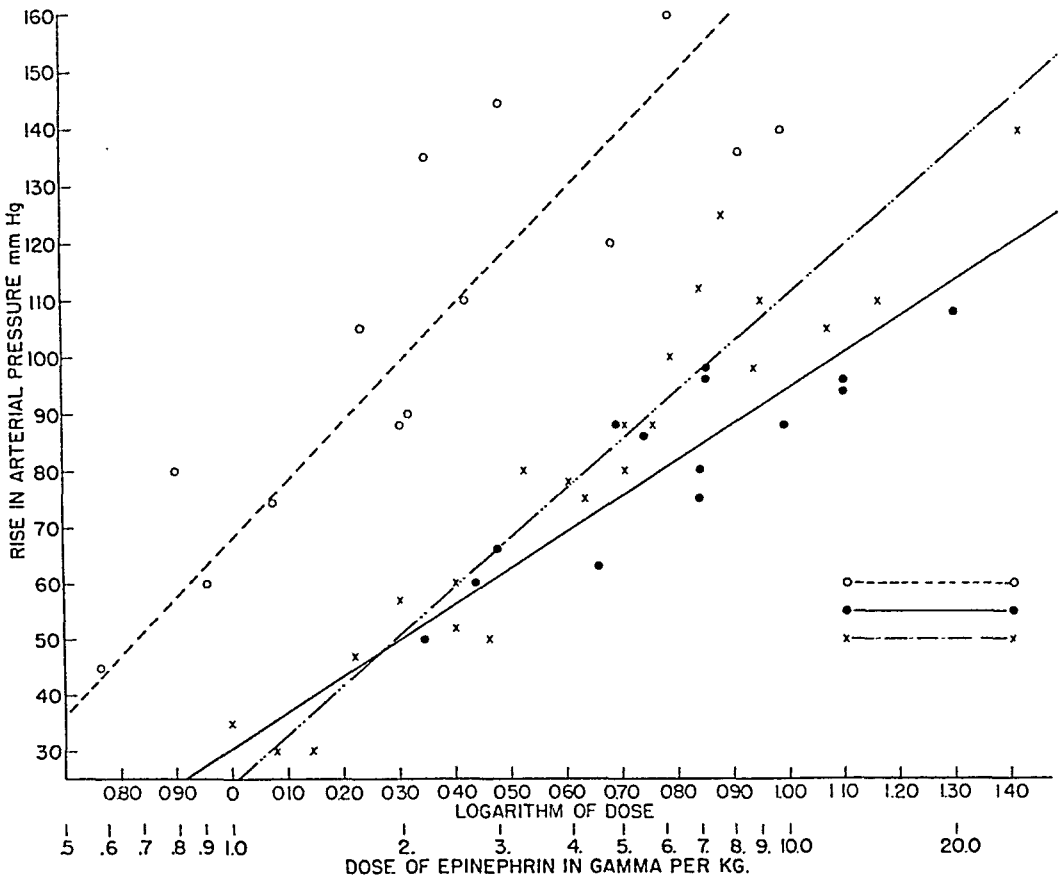
Fig. 4. Relation of rise in blood pressure, in pithed rabbits, to dose of epinephrine injected. Renal hypertensives, 0 and-----; normals, solid circles and line; renal hypertensives pithed 30 hours after nephrectomy, x and –.... Renal hypertensive rabbits lose their sensitivity to epinephrine after the kidney is removed and arterial pressure falls to normal.

much greater in rabbits which were hypertensive at the time they were pithed. The average sensitivity to epinephrine (k in the formula: rise in pressure $= k$ Log. of dose $+ a$), was 60 per cent greater in such rabbits than in normals or those nephrectomized 30 hours previously. Stated in another way it takes three times as much epinephrine to produce a rise of 50 mm. in the pithed normal rabbit as it does in pithed hypertensives and five times as much to produce a rise of 100 mm. In the three pairs of ani-

mals in which responses to pituitrin were noted, after pithing, the sensitivity of controls and hypertensives was the same. In unanesthetized intact rabbits with renal hypertension, pressor response to pituitrin is greater than in controls (Brown et al., 1939; Page and Ogden).

DISCUSSION. It can not be denied that pithing is likely to produce shock, but it does not interfere with response to any of the known peripherally active vasoconstrictors, and often is used to provide unusually sensitive preparations for demonstrating their action (Page and Helmer, 1940). In pithed rabbits given intravenous acacia or Locke's solution diuresis was marked, and it is difficult to see how a peripherally acting vasoconstrictor of renal origin could be inhibited even by a shocking procedure which does not diminish the effect of epinephrine, renin or pitressin, and does not inhibit renal function. On the other hand, study of the rôle of the sympathetic system or of the spinal cord can only be analyzed by means of such acute experiments, since the body possesses means for gradually reëstablishing the control of the central nervous system over the vascular bed when these normal pathways are destroyed (Brown and Maycock, 1940; Nowak and Walker, 1939). When the cord below C4 is destroyed, or the sympathetic system gradually ablated, normal blood pressure is restored and maintained. Renal hypertension less severe than in intact animals can then be produced, but since the mechanism by which normal pressure is maintained in animals with severed nerve tracts is unknown, such experiments cast little light on the mechanism of renal hypertension. The acute experiment does serve to show that the nervous system is as important in maintaining hypertension as it is in maintaining normal pressure levels.

Such observations suggest that an important part of the action of the humoral agent causing renal hypertension is to modify the "set" of the vasomotor mechanism, just as substances from inflamed or necrotic tissue modify the "set" of the heat regulating center. The same mechanisms are used to conserve heat or liberate extra heat in order to maintain the temperature at 40° in typhoid fever as are used to hold it at 37° under normal conditions. Since hypertensive animals and patients are able to regulate flow to the tissues, for temperature regulation or in response to exercise or nervous stimuli, in the same way as do normals, it seems not improbable that the normal regulatory mechanism continues to function, unaffected save for a change in the level at which the controlling center holds the mean arterial pressure.

At the time when the observations on sympathectomy or chronic cord damage in relation to renal hypertension were reported it was not realized that the nervous system could regain control of the vasomotor and cardioaccelerator mechanism after a lapse of days or weeks, and it was reasonable

to assume that the central nervous system and sympathetics were relatively unimportant in causation of renal hypertension. The occurrence of a fall in pressure when the spinal cord is severed in sympathectomized cats (Brown and Maycock, 1940) and of a gradual rise after section of the carotid and aortic nerves in sympathectomized dogs (Nowak and Walker, 1939) are examples of the persistence of central influences after section of the normal vasomotor pathways. The immediate but usually transient fall of pressure after splanchnic nerve section in hypertensive patients, and the acute experiments here described prove that the sympathetic and central nervous systems are important links in the mechanism causing chronic hypertension, renal or nonrenal in origin.

When it was observed in these experiments that pithed hypertensive animals are much more sensitive than pithed control rabbits, it was suspected that arterial hypertrophy might account for this, as the animals had been hypertensive for three to six months. For this reason sensitivity was studied after nephrectomy in animals which had been hypertensive for even longer periods, five to eight months. Under these conditions sensitivity fell to the normal level, as did mean carotid pressure, within 30 hours after removing the kidney which had caused the hypertension. This leaves no doubt that a substance which sensitizes to epinephrine and perhaps to vasomotor nerve stimuli is present in renal hypertension. Such a substance, acting only in this way, can scarcely cause hypertension, for if the moderator nerve reflexes were unaffected any rise in pressure would simply cut down the outflow of vasoconstrictor impulses, resulting in maintaining normal pressure with a lower rate of vasomotor tonic stimulation. Such a sensitization, along with an action on the vasomotor regulatory center raising the level at which tonic impulses were inhibited, would permit maintenance of hypertension without great increase in the rate of discharge from the center and ganglion cells along the vasomotor path.

SUMMARY

On pithing the central nervous system of rabbits those with renal hypertension have a rapid fall of blood pressure to as low a level as normal controls. Even holding venous pressure at high levels by intravenous infusion of acacia-Locke's solution fails to restore normal arterial pressure in these animals.

In pithed rabbits a rise in arterial pressure is easily evoked with epinephrine; the response is greater in those which have had renal hypertension for several months, but not in hypertensive rabbits nephrectomized 30 hours before pithing. No rise in venous pressure precedes the rise in arterial pressure. Renin, like epinephrine, causes a rise in arterial pressure in pithed rabbits, and thus differs from the humoral agent causing chronic

renal hypertension. The latter apparently changes the reaction of the vasomotor center so as to maintain pressure at high levels. It also increases the sensitivity of the arterial response to epinephrine.

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VARIABILITY OF BLOOD pH AND ITS ASSOCIATION WITH METEOROLOGICAL FACTORS

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The importance of the hydrogen ion concentration of the blood for a great variety of biological phenomena has long been recognized and observations on the normal human have revealed a range that is presumably rather narrow (1, 2, 3) though the reports are rather conflicting because in many instances seasonal factors have been neglected and also because of difference in technique. Variations that were associated with weather changes (barometric pressure) (4) as well as seasonal changes (5) have been reported by Petersen in humans. Studies on the influence of altitude and temperature on pH (7) have appeared from time to time, as well as observations extending over short periods. There has been a lack of controlled frequent observations on pH extending over prolonged periods.

Because of the difficulty of controlling "normal" human subjects over long periods of time, a study was first undertaken on the variations in pH over a period of a year in a group of "normal" dogs and compared with a series of simultaneous determinations made on groups of humans in order to determine whether any consistent similarities or differences were present.

EXPERIMENTAL. Employing a group of six dogs of approximately the same size and weight, blood was drawn from each dog in rotation on successive days in order to include in our determinations a picture of the daily variations that have been previously reported (4, 5), and to avoid the possibility of thrombosis and occlusion in the superficial veins which are likely to occur in a dog following daily venous punctures over prolonged periods. The animals were given a standard diet throughout the experiment.

The pH determinations were made on blood plasma according to the glass electrode method. The buffer solution was compared at weekly intervals with a standard pH solution. To assure constancy of our standard pH solutions they were checked every six weeks against numerous precision hydrogen electrode assemblies. The blood having been drawn under oil with a minimum of trauma and transferred under oil into constricted tubes, containing three drops of 20 per cent potassium oxalate, (the reaction of which was repeatedly tested throughout the experiment),

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the determinations were made within fifteen minutes following all of the
precautions recommended to insure accurate readings with our apparatus.
The readings at room temperature were corrected after the method de-
scribed by Myers and Muntwyler (6).   Our readings were checked at
intervals against readings made immediately following the withdrawal of

TABLE 1

Mean pH and standard errors for each dog

DOG	MEAN pH	STANDARD ERROR
1	7.43	±0.02
2	7.46	±0.02
3	7.47	±0.02
4	7.46	±0.02
6	7.47	±0.02
7	7.45	±0.02
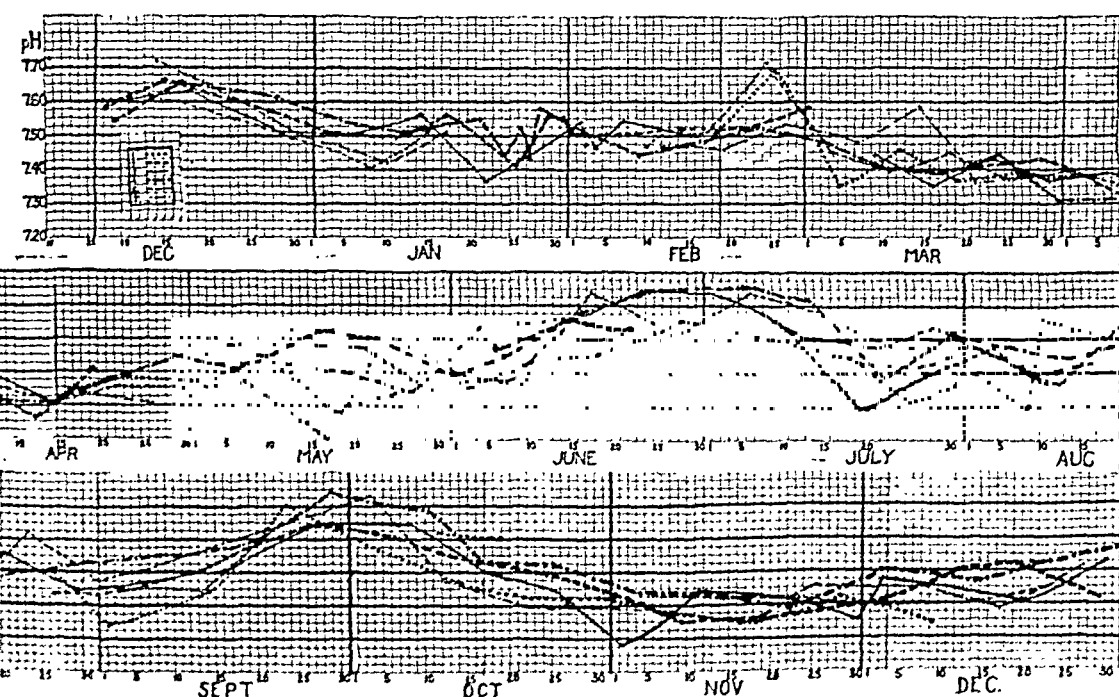
Fig. 1. A continuous record of pH determinations carried out on 6 dogs over a
13-month period.   Each dog being taken in rotation on successive days of the week,
and the weekly readings connected by a different line for each animal.

blood from the veins using a micro-chamber electrode.   The Coleman pH
electrometer was used which has a standard error of ± 0.01 pH.

   After a period of training the pH determinations of the dogs were started
at the beginning of December, 1937 and extended through December,
1938.   The average pH and the standard errors were alike.

Figure 1 shows the character and the degree of fluctuations of the individual readings for each dog. The weekly readings for each dog were connected by a separate line; the relationships between the lines throughout the thirteen month period may be observed in the chart. In general, they present a similar pattern of variations throughout a relatively wide range. Some of the individual variations may be accounted for by the manner in which the readings were rotated from day to day. It has been pointed out (4, 5) that day by day determinations reveal a distinct variation of pH level associated with meteorological aberrations.

Groups of four to six humans were studied daily from January through December 1938. In general, the readings for each individual presented a similar pattern, rising and falling more or less synchronously.
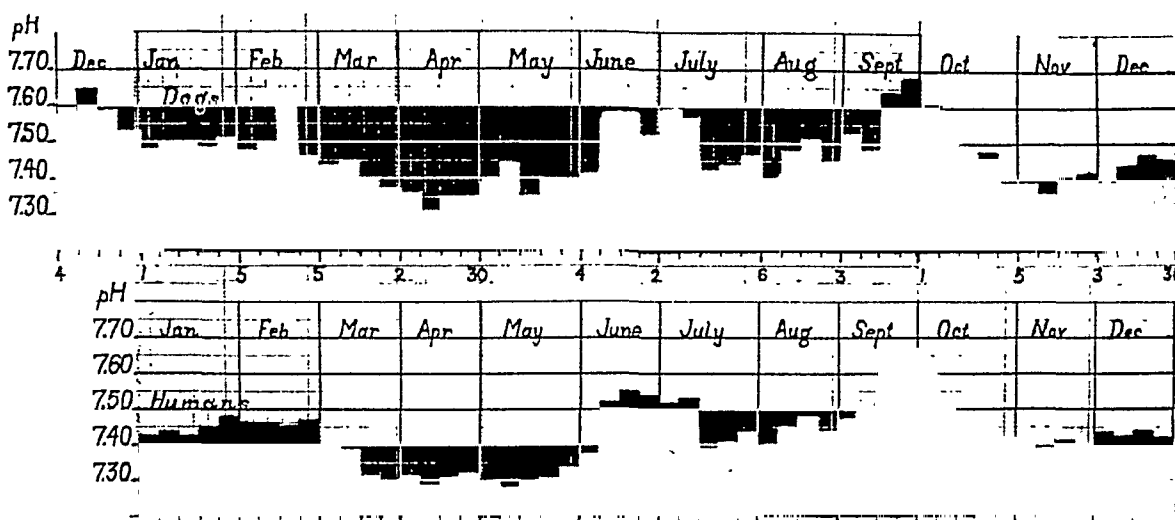
Fig. 2. Weekly average pH values for dogs (above) and humans (below).

Figure 2 shows the average pH level of the weekly cycle. The upper blocks of black represent the weekly average pH for the six dogs while the lower blocks represent the composite weekly average for the humans. Comparing the two series of blocks the close similarity may be seen, and statistically the correlation coefficient is high, being + 0.87.

From figures 1 and 2 it is evident that the pH of the various individuals (fig. 1) move in approximately the same manner. Further, the pH of two different species move in much the same fashion throughout the year (fig. 2). It is clear that at least a part of the variation of pH is determined, either directly or indirectly, by one or more factors to which all individuals studied (of either species) are exposed simultaneously. In endeavoring to determine what factors may account for these similar fluctuations in pH levels, a number of factors, both external and internal, were considered. Since the diets of the two species differed, the diet of the dogs being uniform through the year, the possibility that the fluctuations in pH might be

wholly due to seasonal differences in diet must be discarded. It was decided to investigate, if possible, the relationships between the several indices of the weather and the pH for both the humans and the dogs. Previous studies by Petersen (4) have given evidence of the association of the variations of a number of chemical constituents of the blood with the variations of barometric pressure and daily temperature.

The daily mean temperature, which was averaged to attain a single weekly figure, was obtained from the *Monthly Summary* of the U. S. Weather Bureau's Chicago Station. The daily 7 a.m. and 7 p.m. barometric pressure, which was likewise averaged to yield a weekly series, was obtained from the unpublished records of the Chicago Station.

The nature of the influence of the weather on pH, if it exists, is probably complicated and it may be necessary to start with certain simplifying assumptions regarding the possible functional relations. However, the nature of the functional relationship may be detected somewhat by an empirical study of the statistics. As each week has been considered as a discrete observation, some of the continuity has probably been lost in the pH series. Turning to the data itself, it has been observed that the pH was somewhat more stable than the barometric pressure, which at times showed great variability. For this reason it was decided to compute a three week moving average of the pH, barometric pressure, and temperature data.

By plotting pH against barometric pressure and temperature it was possible to judge the type of the functional relationship which exists between these variables. The charts clearly indicated that an arithmetical relationship was not involved, for the points tended to scatter in a fan-shaped manner. Conversion to logarithms seemed to reduce the scattering of the points in a manner which made their distribution relatively homoscedastic. Thus it appeared that it was a percentage change in pH which was associated with a percentage change in the barometric pressure. No definite conclusions could be reached concerning the relationship between pH and mean temperature and so the mean temperature was left on an arithmetic scale. In order to bring out most clearly the relationships, it was necessary to code the variables in the following manner: the 7.00 was dropped from the pH readings as the variation was in the last two figures, and then the reduced readings were multiplied by 4.00 to achieve a better transformation in logarithms. The barometric pressure was coded by subtracting 29.00 inches. For example, instead of 30.10 inches the figure of 1.10 inches was used. The mean temperature readings for the week were left untouched.

As the above codings were made prior to the transformation to logarithms, the various observations received somewhat different weights than those which a direct transformation would have given. The coding

also becomes a part of the description of the functional relationships between variables. The proposed mathematical function relating the pH to barometric pressure and mean temperature then becomes by the above adjustments:

$$\text{Log} [4.0(\text{pH}-7.00)] = \alpha + \beta \cdot \text{Log} [\text{Bar. P.}] + \delta [\text{Temp.}] \quad \text{(Ia)}$$

or as an exponential:

$$\text{pH} = 7.00 + \frac{1}{4.0} [(10)^{\alpha}] \cdot [\text{Bar. P.}^{\beta}] \cdot [(10)^{\text{Temp.} \delta}] \quad \text{(Ib)}$$

The statistics α, β and δ may be found by the method of least squares.

The Pearsonian product moment correlation coefficients for the coded variables were computed and the results between the pH readings, temperature and weather, are shown in table 2. The intercorrelation between the log of barometric pressure and mean temperature is -0.38.

Before answering the question which immediately suggests itself of whether these correlations are significantly different from zero, certain

TABLE 2

Zero order correlation coefficients

	BAROM. PRES.	TEMPERATURE
Human pH......................	+0.60	+0.18
Dog pH........................	+0.52	+0.19

reservations must be considered. In this instance we are not dealing with conditions of simple sampling for which the usual tests of significance were devised; the observations were not chosen at random, but are ordered in time; secondly, the data have been smoothed by means of the moving average, decreasing the true number of degrees of freedom available for determining the estimating equation.

A partial compensation for these difficulties may be gained by requiring a somewhat higher level of significance, say, the probability discarding a negative hypothesis (zero correlation) be set at 0.001 instead of 0.01. Further, it is necessary to consider the possibility that factors which could be neglected in the present study because they happened to remain relatively stable over the period studied might vary significantly in some later period. Turning to Fisher's table for testing of correlation coefficients (8), it is seen that if random samples of 50 paired observations each are taken from a universe of zero correlation, the correlation of ± 0.361 or greater is obtained only once in one hundred times. As the correlations with the barometric pressure for the pH of humans is $+0.60$ and for the pH of the dogs is $+0.52$, which are of greater value than the one per cent

level (±0.361), even after considering the above reservations, it seems safe to conclude that the hypothesis that there is no correlation between pH and barometric pressure should be discarded as improbable.

The correlations with temperature and pH (dog, +0.18, and human. +0.19) are not to be considered significantly different from zero, as the 10 per cent level of Fisher's table indicated that a correlation of at least ±0.230 or greater is obtained ten out of one hundred times with random sampling.

The intercorrelation between log of barometric pressure and temperature, of −0.3770, may be considered significantly different from zero. The probability of obtaining correlations which differ as much or more than +0.60 (human) and +0.52 (dog), if they are really drawn from the same universe, is 61 out of one hundred. It is necessary to conclude that there is no significant difference between the correlations.

Because of the importance of the intercorrelations between barometric pressure and mean temperature, it is clearly necessary to consider these two weather indices simultaneously. The parameters required in equations (Ia) and (Ib) are easily found and the estimating equations become: Human:

$$\text{Log } [4.0(\text{pH}-7.00)] = -0.1052$$

$$+ 0.2022 \cdot \text{Log } [\text{Bar. P.}] + 0.002589 \text{ [Temp.]} \quad \text{(IIa)}$$

$$\text{pH} = 7.00 + 0.200 \cdot [\text{Bar. P.}]^{0.2022} \cdot [10]^{\text{Temp. } 0.002589} \quad \text{(IIb)}$$

Dog:

$$\text{Log } [4.0(\text{pH}-7.00) = +0.0323$$

$$+ 0.1379 \cdot \text{Log } [\text{Bar. P.}] + 0.001863 \text{ [Temp.]} \quad \text{(IIa')}$$

$$\text{pH} = 7.00 + 0.268 [\text{Bar. P.}]^{0.1379} \cdot [10]^{\text{Temp. } 0.001863} \quad \text{(IIb')}$$

The relative importance of the barometric pressure and temperature in determining the pH values is not well pictured by the above equations because the barometric pressure and temperature readings are measured in different units. If the variables are converted to units such that the standard deviations are equal, coefficients which are more comparable may be computed. Table 3 gives the *Betas*, or the coefficients in standard units. It is seen that the barometric pressure is of greater importance in the estimation of pH than is the temperature.

The accuracy with which the estimating equations (IIa) and (IIa') predict the values of pH is given by the multiple correlation coefficient. The multiple correlation coefficient for the human pH with barometric pressure and temperature is 0.7383 and for the dog pH is 0.6670. Both of these correlations are significantly different from zero.

The importance of the interplay of barometric pressure and temperature is clearly brought out when we turn to the partial correlations. A partial correlation attempts to give a picture of the effect of one of the independent variables—say, barometric pressure—upon the dependent variable, pH, when the other independent variable or variables, say, temperature, are held constant. It is the statistical approximation to the control of certain important influencing factors in the experiment conducted in a laboratory.

The partial correlations as compared to the zero order correlations (table 2) have in all instances been increased due to the negative correlation between the temperature and barometric pressure. While each of the weather indices tends to affect the pH in a positive fashion, they tend to move in opposite directions resulting in a partial negation of the net effects upon pH.

TABLE 3

Coefficients of estimating equations in standard units

	BAROM. PRES.	TEMPERATURE
Human pH......................	+0.77	+0.47
Dog pH........................	+0.69	+0.45

TABLE 4

Partial correlation coefficients

	BAROM. PRES.	TEMPERATURE
Human pH......................	+0.73	+0.54
Dog pH........................	+0.65	+0.49

All four partial correlations in table 4 can be considered as significantly different from zero as a partial correlation of ±0.37 or greater is obtained only one per cent of the time from a universe of zero correlation (Fisher's table 5, A (8)). The use of partial correlational technique clearly brings out the importance of considering both temperature and barometric pressure and it is seen that temperature does play an important part in the determination of the pH.

Until now it has been assumed that the relationships between the variables as adjusted by transformation into logarithms were necessarily rectilinear; however, it may be well to investigate the possibility of curvelinearity. The method of successive approximation suggested by Ezekial (9) was used. The residuals from the estimating equations are plotted against: first, the barometric pressure; and secondly, against the temperature, correcting the regression if the straight line is not visually the "best fit." If these new regressions still do not satisfy, adjustments are made

again or until the best curvelinear regressions are found. The disadvantage of the method, of course is that in the end no mathematical equation is obtained unless the work of fitting is repeated, assuming another general
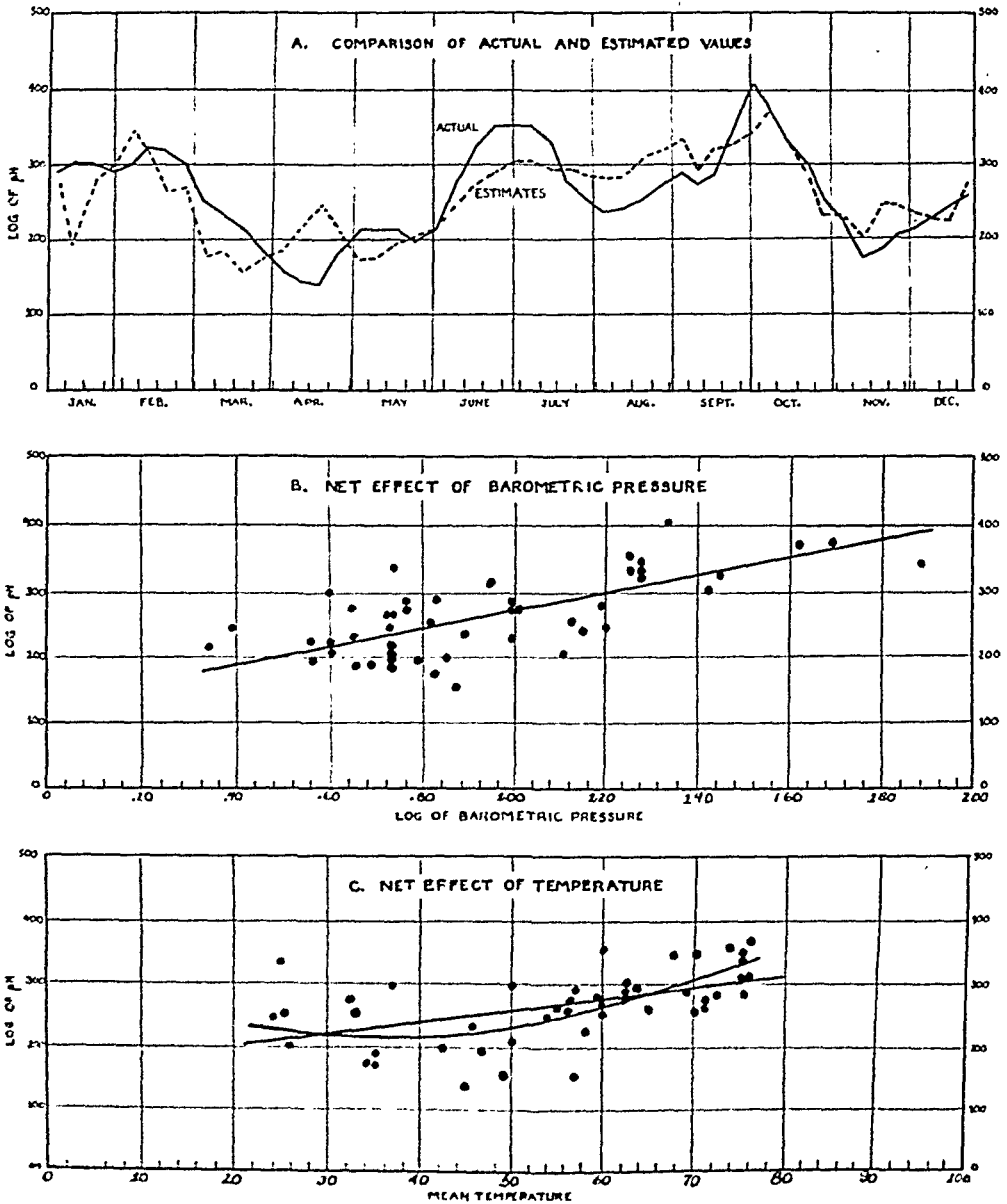
Fig. 3. pH of dogs: section A, comparison of logarithms of actual values and logarithms of pH as estimated from barometric pressure and mean temperature; section B, the net effect of barometric pressure on pH; section C, the net effect of mean temperature on pH.

equation which seems to meet the requirements of the corrections for curvelinearity. Further, there is no adequate way to determine the number of degrees of freedom absorbed by the correction.

In figure 3 are shown the corrections for curvelinearity for the dog pH. Section B shows the net effect of the barometric pressure and clearly indicates that the influence is linear. The lowest section of the figure shows that the assumption of linearity does not hold. As temperature rises from a weekly average of 42° the pH rises at an increasing rate. Below a temperature of 42° the pH seems to rise slightly. The nature of the relationship may be due to the living conditions of the animals, as they were kept in steam-heated buildings and were not exposed to the extreme cold, while during the summer they were not as well protected against excessive heat. It may be that during the coldest days they were living under much warmer conditions than during only moderately cold days, or the possibility of compensating physiological mechanisms must be considered. In section A the actual values of the logarithms of pH as observed are compared to the values estimated from the relations shown in the lower sections of the chart. It is seen that the estimates follow the actual values quite closely. The importance of curvelinearity of the temperature relationship is reflected in the increase of the linear multiple correlation coefficient of 0.6670 to the curvelinear index of multiple correlation of 0.7345.

Figure 4, in turn, shows the similar material for the human pH. The relationship of the barometric pressure and pH is slightly curvelinear as is shown in section B. The pH level rises more rapidly as the barometer moves from lower level than when the increases occur at the higher levels. The deviation from linearity may not be significant and it may be that no real difference exists between the reaction of the pH of the dogs and the pH of the humans to barometric pressure. In section C the relationship of the pH of the humans with temperature shows the same curvelinear tendencies as were discovered for the dogs. A low pH is reached at about 42° with pH increasing more rapidly as temperature rises; slight increases in pH occur with drops in temperature below 42°. The latter phenomenon might also be attributed to our ability to protect ourselves against the cold on the one hand by clothes and warm indoor temperatures, or on the other hand, by a possible compensatory mechanism. In section A will be found the comparison of the actual and estimated values, the correspondence of which is somewhat closer than for the dogs. The importance of the curvelinearity is evidenced by the increase in the linear multiple correlation of 0.7383 to a curvelinear Index of Multiple Correlation of 0.8224. This is an important increase in the "closeness of fit," for the standard error of estimate has dropped from 0.45 to 0.25, or only 25 per cent of the variance of the logarithms of pH is left unexplained as compared to 45 per cent under the assumption of linearity.

DISCUSSION. Because of the high correlation between dogs and humans even with the relatively wide fluctuations in pH—from 7.32 to 7.68—

some of the complex mechanisms involving the pH of each may be briefly considered together.   The pH measured was that of venous blood of an
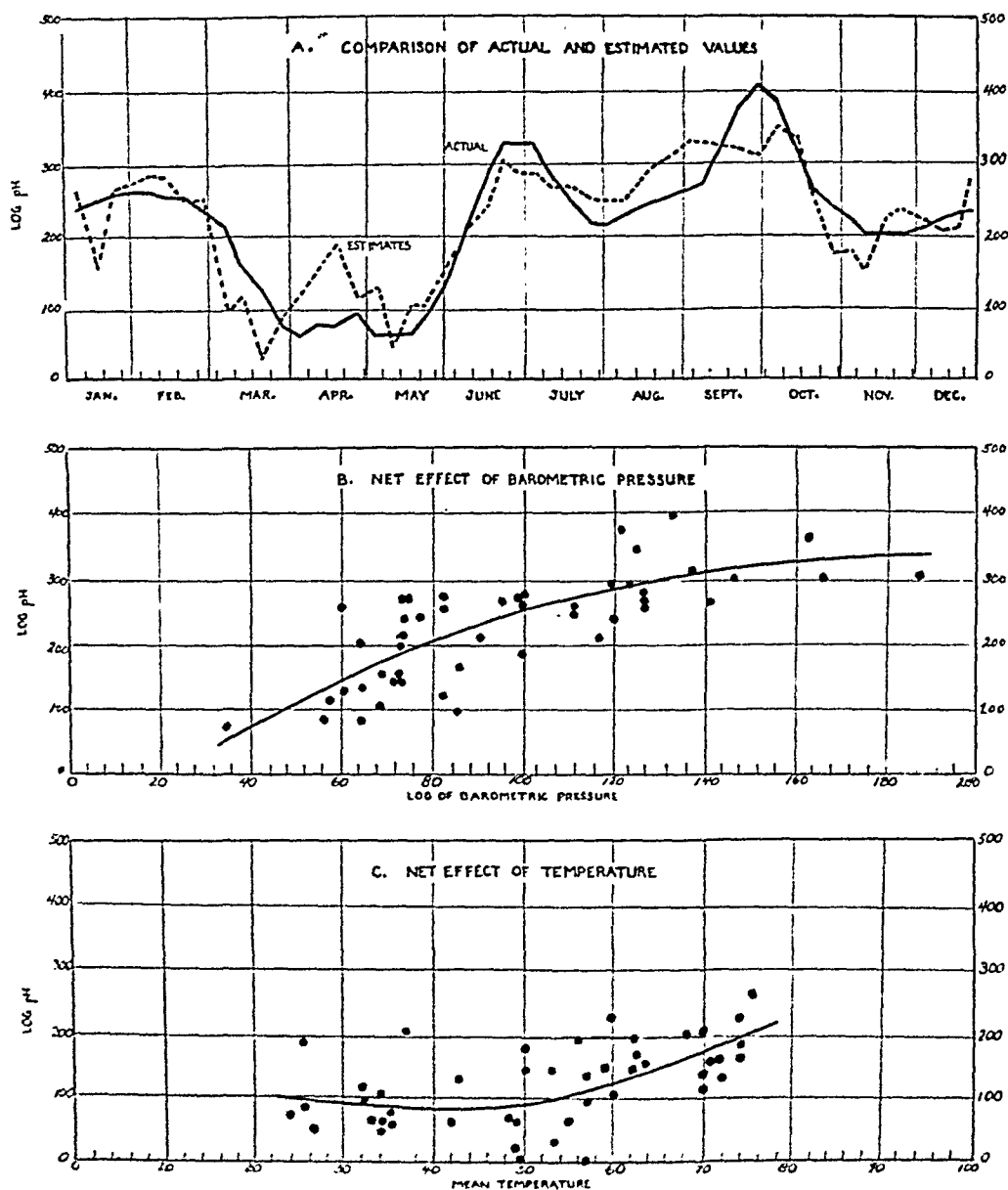
Fig. 4. pH of humans: section A, comparison of logarithms of actual values and logarithms of pH as estimated from barometric pressure and mean temperature; section B, the net effect of barometric pressure on pH; section C, the net effect of mean temperature on pH.

extremity and thus was possibly subject to wider variations than arterial blood.   Since pH is a measure of the hydrogen ion activity (10) in a biologic system at a given time, variations in the buffers (ratio of base to

bicarbonate), the rate of oxidation and reduction, and the rate of elimination of metabolic products will affect the pH. In comparing the CO_2 contents of the blood with the pH a general reciprocal relation was seen. However, the CO_2 content was subject to wide fluctuations without corresponding changes in the pH. The CO_2 content depends upon (7) the amount of available alkali (alkali reserve) and the amount of hemoglobin which, like other acids, competes for the alkali, as well as upon the ratio of production to elimination. Increasing the environmental temperature lowers the CO_2 content (7) and inversely tends to raise the pH. Although temperature and barometric pressure are acting simultaneously (and the importance of their interplay has been demonstrated above), for purposes of simplification and clarity it may be well to discuss them separately.

It was seen that the pH levels tended to rise at an increasing rate as the temperature rises above 42°, however, with the temperature falling below 42° the pH seems to rise slightly. While the temperature has a definite effect on the pH, the effect of temperature was found to be not as great as barometric pressure which may be due to the ability of the organism to compensate readily for changes such as variation in the CO_2 content, for example, through the respiratory mechanism. Furthermore, as barometric pressure and temperature tend to move in opposite directions, some of the effects of temperature appear to be partially negated. However, at the higher levels of temperature which are associated with an increased respiratory rate, more CO_2 is blown off and the pH rises at an increasing rate.

Changes in barometric pressure are statistically definitely more important than changes in temperature. The relationship appears to be a linear one with a leveling off of pH at the high pressure readings. The distinctly higher correlation between the barometric pressure and pH is probably due to a more complex mechanism perhaps involving such factors as circulatory changes (5), the oxygen tension of the blood (increasing pressure increases oxygen content) and the compensatory effects at various levels and the greater release of acid metabolites associated with anoxia. Changes in pH in turn, have been shown to influence the oxidation rates of various organs and tissues with increasing rates at lower pH (11, 12, 13, 14).

Suggestive among the factors which may possibly influence the pH are variations in the energy production. Petersen (5) has observed changes in B.M.R. and pH, associated with meteorological alterations. Ritzmann and Benedict (15) have shown variations in B.M.R. which they believed might be due to seasonal changes in the weather. Correspondingly distinct seasonal variations in the organic iodine content in the thyroid of domestic animals has been recently reported by Seidell and Fenger (16).

With abrupt changes in temperature and barometric pressure (polar fronts), the importance of circulatory phenomena (vascular spasm and relaxation) have been brought out (5), and these have been associated with fluctuations in the blood chemistry, among them pH (rising and falling pH). When meteorological stimuli were either very pronounced or frequent, summation effects were observed. Statistically significant daily variations in pH have also been reported by Shock and Hastings (3) and the data showed general trends over a period of several days.

Considerable controversy has arisen in discussing seasonal influences, due to failures to duplicate results. That this must occur is logical, for one spring may not be like another spring and one May not like another May. The temperature may be low and the barometric pressure high, or they may be unstable one year and more stable the next. One cannot compare corresponding months of different years and consider them similar only because of their order in time. On such a basis failures to duplicate results cannot be considered as discounting climatic influence. In comparing pH values collected in previous years (5) in this laboratory with those presented in this paper, definite differences were encountered. In a number of years, for example, the levels in the spring were low—in some there was no decline—while in others they were relatively high.

SUMMARY

1. A high correlation was found in the pattern and in the degree of variation of the pH of the blood among a group of dogs studied over a period of one year.

2. A high statistical correlation was obtained in comparing the continuous pH values in a group of dogs with those of a group of humans for a period of one year.

3. A relatively wide degree of fluctuation was observed in both humans and dogs. In man the weekly averages varied from 7.28 to 7.68; while in the dogs they varied from 7.32 to 7.68.

4. In comparing the pH and the weather, statistically significant coefficients were found with barometric pressure and temperature, with a distinctly higher correlation being found for barometric pressure than for temperature. Increases in pH were associated with increases in barometric pressure. On the other hand, the relationship between pH and temperature was not as marked and only at higher temperatures were increases in pH definitely associated with increases in temperature. In attempting to evaluate seasonal influences varying results were found, depending upon the weather indices discussed.

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DEMONSTRATION OF THE LIBERATION OF RENIN INTO THE BLOOD STREAM FROM KIDNEYS OF ANIMALS MADE HYPERTENSIVE BY CELLOPHANE PERINEPHRITIS

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It has been shown that the perinephric reaction to cellophane and silk produces a constricting hull around the parenchyma of the kidneys which causes persistent arterial hypertension (1). The renin-angiotonin vasopressor system is believed to be involved in the genesis of this type of experimental hypertension. In short, the results of studies on this system have shown that renin is not itself a pressor substance because extensive purification yields an enzyme-like substance without vasoconstrictor action in organs perfused with Ringer's solution (2). Fractionation of blood produces a substance (renin-activator) which when added to the purified renin caused the reaction mixture to assume powerful vasoconstrictor properties (3). The product of the interaction of renin and renin-activator was isolated, crystallized and named angiotonin (4, 5). Angiotonin also has an activator separable from blood (6). The kidneys form inhibitors to both renin and angiotonin (7).

With these observations in mind, it was obviously important to demonstrate that the kidneys of hypertensive animals did in fact liberate renin and in amounts greater than from normal ones. Employing normal kidneys perfused with blood, Kohlstaedt and Page (8) found that reduction of pulse pressure led to the liberation of a substance from the renal vein which reacted with purified renin-activator to produce strong vasoconstriction. Since the reaction between renin and renin-activator appears to be specific, there was good reason to believe that angiotonin had been produced by the interaction of renin liberated from the perfused kidney and renin-activator added to the blood from the renal vein. Kidneys perfused at normal pulse pressure liberated only small amounts or no renin.

We have now studied the blood from normal kidneys and kidneys with their parenchyma constricted by the fibro-collagenous hull incited by cellophane or silk perinephritis. Since it has been shown that injection of renin quickly exhausts the supply of renin-activator present in normal blood (7), it would be anticipated that, if renin were liberated in large

amounts from the kidneys, blood from the renal vein would contain little renin-activator and excess renin. To demonstrate this, renin-activator was added to the plasma, the mixture incubated, and tested for angiotonin by means of the perfused rabbit's ear preparation.

METHODS. Hypertension was produced by application of cellophane or silk to the parenchyma of the kidneys of normal and uninephrectomized dogs. In some animals the kidneys were transplanted up under the skin by the method of Page and Corcoran (9) to facilitate drawing of renal vein blood.

After severe hypertension was established, renal venous and femoral arterial and venous blood samples were drawn, heparinized, and, after centrifuging, the plasma separated. The renin content of the plasma was ascertained by circulating a mixture of plasma with renin-activator, prepared by the method of Kohlstaedt, Page, and Helmer (10), through a rabbit's ear perfused by pulsatile pressure with Ringer-Locke's solution. Usually 0.2 cc. of plasma was mixed with activator in the proportion of 1 to 17, incubated at 25°C. for 10 minutes and then injected into the perfusing fluid. The plasma was also tested for its angiotonin- and renin-activator content by addition respectively of angiotonin and renin.

RESULTS. What we believe to be examples of typical experiments are given in table 1.

Addition of renin-activator to plasma from the renal vein of normal dogs causes only moderate constriction, when the same amount of plasma or renin-activator alone causes none. But plasma from the renal vein of a hypertensive dog, provided the hypertension is not malignant, on addition of activator causes 2 to 6 times as much constriction as normal. There is no great difference between femoral arterial and venous blood though often femoral blood appears to contain slightly more renin. Neither contain amounts of renin comparable with that in the renal vein.

Addition of renin to plasma in the proportion of 1 part to 17 parts of plasma, produced a mixture with marked constrictor properties, the renal vein blood usually being somewhat less active than femoral venous or arterial blood. The femoral arterial blood from hypertensive dogs produced greater constriction than femoral blood from normal dogs.

Addition of angiotonin to plasma caused marked vasoconstriction regardless of the origin of the blood, but femoral arterial blood from hypertensive animals gave distinctly greater than normal responses, with femoral venous blood next in order of potency.

It is of especial interest that, in 3 dogs with the syndrome of malignant hypertension, addition of renin-activator to plasma from the renal vein caused little vasoconstriction, nor did addition of renin or angiotonin. Large amounts of renin were found in the renal vein of one dog early in the course of malignant hypertension.

TABLE 1

The effect of addition of renin-activator, renin, and angiotonin on the vasoconstrictor
properties of plasma from renal and other blood vessels when perfused
through an isolated rabbit's ear

DOG NO.	B.P.	ORIGIN OF BLOOD*	RENIN-ACTI- VATOR ADDED		RENIN ADDED		ANGIOTONIN ADDED		CONDITIONS OF EXPERIMENT
			Reduction of flow through ear vessels						
	mm. Hg		*min-utes*	*per cent*	*min-utes*	*per cent*	*min-utes*	*per cent*	
1	126	R. R. V.	½	20	1¼	54	3	62	Both kidneys explanted.
		F. V.	¼	20	1½	60	3	70	No anesthetic
		F. A.	¼	23	2	63	4	64	
1	170	L. R. V.	7	82	½	12	½	20	Both kidneys explanted.
		R. R. V.	½	20	1	38	1	28	No anesthesia. Left
		F. V.	1	49	1	50	1	32	renal artery con-
		F. A.	¾	31	1½	44	1½	39	stricted 10 days before
1	178	L. R. V.	5½	84	4	47	4	51	Same 3 days later
		R. R. V.	3	49	7	65	4	46	
		F. V.	1½	47	10	81	10	90	
		F. A.	1½	40	10	76	12	77	
1	182	L. R. V.	8	94					Same 4 days later
		R. R. V.	½	28					
1	166	L. R. V.	3	72	1	47			Same 5 days later and
		F. V.	½	18	2½	56			after right nephrec-
		F. A.			3	64			tomy
1	166	L. R. V.	3	81	1	16	1	32	7 days after right ne-
		F. V.	2	72	1	60	3	59	phrectomy
		F. A.	½	21	2½	79	5	65	
1	180	L. R. V.	3	57			½	17	13 days after nephrec-
		F. V.	1½	63			½	26	tomy. Signs of ma-
		F. A.	¼	22			1	47	lignant hypertension. B.U.N. = 56 mgm.
1	189	L. R. V.	2½	57			¾	24	14 days after nephrec-
		F. V.	½	26			½	26	tomy. Anuria
		F. A.	0	0			1	39	
1	192	L. R. V.	2½	42	¾	34	½	25	15 days after nephrec-
		F. A.	½	10	1¼	39	1¼	51	tomy. Just after loosening clamp. B.U.N. = 133 mgm. Died 2 days later

TABLE 1—*Continued*

DOG NO.	B.P.	ORIGIN OF BLOOD*	RENIN-ACTIVATOR ADDED		RENIN ADDED		ANGIOTONIN ADDED		CONDITIONS OF EXPERIMENT
	mm. Hg		*minutes*	*per cent*	*minutes*	*per cent*	*minutes*	*per cent*	
									Malignant hypertension
2	182	R. R. V.	8	94	$\frac{1}{2}$	33			Lt. nephrectomy + Rt. silk
		F. A.	2	46	1	39			Malignant hypertension
		Inferior vena cava 3 cm. above renal veins	5	68	1	43			
		Inferior vena cava above hepatic veins	3	61		43			
3	126	L. R. V.	$2\frac{1}{2}$	54					Malignant hypertension. Rt. nephrectomy + silk on Lt. kidney. B.U.N. = 32.0. Retinal detachment and hemorrhages obser. 2 days before. 4 days before B.P. = 210 mm. Hg
		F. V.	$\frac{1}{4}$	14					
		F. A.	$\frac{1}{2}$	17					
4	140	L. R. V.	$1\frac{1}{2}$	30	1	31	1	36	Right + left silk. Malignant hypertension
		R. R. V.	1	29	$2\frac{1}{2}$	54	$1\frac{1}{2}$	57	
		F. A.	0	0	2	47	$1\frac{1}{2}$	43	
5	142	F. A.			$1\frac{1}{2}$	20	1	12	Malignant hypertension
6	202	F. A.			$\frac{1}{2}$	20	$\frac{1}{4}$	29	Malignant hypertension. B.U.N. = 21.5 mgm.
7	224	F. A.					$\frac{1}{2}$	34	Malignant hypertension. B.U.N. = 75.0 mgm.
8	88	F. A.			$\frac{1}{2}$	40	$1\frac{1}{2}$	44	Both kidneys in cellophane. Malignant hypertension. B.U.N. = 90.8 mgm.
									Normal
9		F. A.			3	69	2	46	Normal dog
10		F. A.			2	40	1	37	Normal dog
11		F. A.			2	30	1	37	Normal dog
					1	40	1	41	

TABLE 1—*Concluded*

DOG NO.	B.P.	ORIGIN OF BLOOD*	RENIN-ACTIVATOR ADDED		RENIN ADDED		ANGIOTONIN ADDED		CONDITIONS OF EXPERIMENT
	mm. Hg		*minutes*	*per cent*	*minutes*	*per cent*	*minutes*	*per cent*	

Normal—*Continued*

DOG NO.	B.P.	ORIGIN OF BLOOD*	RENIN-ACTIVATOR ADDED		RENIN ADDED		ANGIOTONIN ADDED		CONDITIONS OF EXPERIMENT
12	120	L. R. V.	1	68	$\frac{3}{4}$	26	$2\frac{1}{2}$	57	Normal dog under pento-
		R. R. V.	$\frac{1}{2}$	30	$1\frac{1}{2}$	55			barbital anesthesia
		F. V.	$\frac{1}{4}$	46	$1\frac{1}{2}$	39	3	61	
		F. A.	$\frac{1}{2}$	18	2	43	3	64	
13	130	L. R. V.	$\frac{1}{4}$	11	1	41	$1\frac{1}{4}$	47	Normal dog under pento-
		F. V.	$\frac{3}{4}$	19	$1\frac{1}{2}$	58	1	68	barbital anesthesia
		F. A.	$\frac{3}{4}$	15	$1\frac{1}{2}$	61	1	52	
14	240	L. R. V.	$\frac{1}{2}$	31	2	70			Normal dog under pento-
		R. R. V.	$\frac{3}{4}$	40					barbital anesthesia
		F. V.	$\frac{1}{2}$	39	2	65			
		F. A.	$\frac{1}{2}$	48	2	78			

Renal hypertension

DOG NO.	B.P.	ORIGIN OF BLOOD*	RENIN-ACTIVATOR ADDED		RENIN ADDED		ANGIOTONIN ADDED		CONDITIONS OF EXPERIMENT
15	182	L. R. V.	$2\frac{3}{4}$	79	$\frac{1}{2}$	18	$1\frac{1}{4}$	54	Both kidneys in cello-
		F. V.	$\frac{1}{2}$	20	2	47	4	79	phane. Anesthetized
		F. A.	$\frac{1}{2}$	22	5	72	$3\frac{1}{2}$	71	with pentobarbital
16	180	L. R. V.	$1\frac{1}{4}$	46	2	70			Both kidneys in cello-
		F. V.	1	40	2	72			phane
		F. A.	1	42	$2\frac{1}{2}$	75	$2\frac{1}{2}$	58	
17	182	L. R. V.	5	69	1	29	1	41	Rt. nephrectomy and
		F. V.	$1\frac{1}{2}$	32	1	32	$1\frac{1}{2}$	47	Lt. kidney in silk
		F. A.	1	34	3	47	2	48	
18	200	L. R. V.			$\frac{1}{2}$	21			Rt. nephrectomy and
		F. V.			$2\frac{1}{2}$	48			Lt. kidney in silk.
		F. A.			$5\frac{1}{2}$	79			Under pentobarbital
									anesthesia
19	172	R. R. V.	$3\frac{1}{2}$	78	$1\frac{1}{2}$	61	2	47	Rt. kidney explanted
		R. R. A.	1	46					Lt. nephrectomy. Rt.
		F. V.	$1\frac{1}{2}$	49	$1\frac{1}{2}$	57	3	51	renal artery con-
		F. A.	1	54	$4\frac{1}{2}$	74	4	68	stricted 3 days before
19	170	R. V.	8	94	3	64			Same 7 days after con-
		F. V.	$2\frac{1}{2}$	47	$2\frac{1}{2}$	59			striction

* L. R. V. = left renal vein, R. R. V. = right renal vein, F. V. = femoral vein, F. A. = femoral artery.

DISCUSSION.   From these experiments it appears that the demonstration of renin in blood depends on the presence of sufficient renin-activator with which it can react to form angiotonin.   If this is true, it is understandable why many attempts to show that renin is liberated into the renal vein blood have failed.

When renin is injected in large amounts into animals, at least two reactions are provoked and either one or both may lead to neutralization of the pressor action of the renin (7).   The first of these is exhaustion of renin-activator which is demonstrable in perfused isolated organs.   The second is liberation of an inhibitor or anti-pressor substance from the kidneys and muscle.   In normal animals the amount of inhibitor to both renin and angiotonin appears to be high, in the hypertensive animal much less, and in the nephrectomized animal it is almost lacking (6).   This suggests that heightened susceptibility to angiotonin may be a vital circumstance for the development of persistent arterial hypertension.

When normal kidneys are perfused at normal pulse pressure, little or no renin is demonstrable in the renal vein blood (8) but if the pulse pressure is reduced, large amounts are liberated as shown by the fact that addition of renin-activator causes marked vasoconstriction when the mixture of renal vein plasma and activator are perfused through a rabbit's ear.   We believe that liberation of renin in increased amounts has now been demonstrated from kidneys of animals made hypertensive by perinephritis, and in two experiments, by constricting the renal artery (Goldblatt).   The amount of renin appears to diminish rapidly in its course through the circulation and by the time the blood has reached the femoral artery it contains at most only slightly increased amounts.   This may be explained on the basis of the reaction of renin with renin-activator to produce angiotonin (4, 5).

The amount of renin-activator in femoral arterial and venous blood apparently increases in hypertension (10) and is slightly decreased in renal vein blood.   This may be due either to an actual increase in production of activator, to decrease in the amount of inhibition, or both.   Therefore, until it is possible to distinguish between increased amounts of activator and decreased amounts of inhibitor, no exact description can be given.

Angiotonin-activator is present in the blood of both normal and hypertensive animals regardless of source, but greater than normal amounts may be found in femoral arterial and venous blood of hypertensive animals.

The animals exhibiting the syndrome of malignant hypertension appear to be characterized by the fact that addition of neither renin nor angiotonin to plasma from the femoral vessels causes marked vasoconstriction.   Since addition of activator does not greatly augment the vasoconstrictor action of the plasma, it may be surmised that the inhibitor content of the blood is elevated.   In one animal (no. 2) relatively early in the course of the disease, addition of renin-activator to plasma from the renal vein caused

marked vasoconstriction, indicating liberation of renin. The plasma from the renal veins from 3 others (nos. 1, 3, 4) exhibited little more renin than normal. Femoral arterial blood was low normal or distinctly subnormal in its angiotonin- and renin-activator content.

CONCLUSIONS

1. Renin is liberated into the renal vein in increased amounts by the kidneys of dogs made hypertensive by cellophane or silk perinephritis, and by clamping the renal artery. Most of it disappears by the time the blood has reached the femoral artery.

2. Renin-activator is decreased in the blood from the renal vein and is increased in hypertensive animals when the femoral artery is reached.

3. Angiotonin-activator is not greatly decreased in the renal vein blood but may be increased in the femoral arterial blood in hypertensive animals.

4. Early in the course of malignant hypertension, large amounts of renin are liberated by the kidneys (1 experiment). Later, both angiotonin-activator and renin-activator are greatly reduced or sufficient inhibitor is formed to abolish the reaction between them and angiotonin or renin (7 experiments).

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DIFFERENCE IN THE ACTIVATING EFFECT OF NORMAL AND HYPERTENSIVE PLASMA ON INTESTINAL SEGMENTS TREATED WITH RENIN

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It has been shown by Kohlstaedt, Helmer and Page (1938) that renin itself is not a pressor substance but when it reacts with an activator contained in blood, prompt vasoconstrictor properties are manifest. This is due, as shown by Page and Helmer (1939), to formation of a heat-stable substance which they have called angiotonin and from which crystalline derivatives have been prepared.

With the hope that some relatively simple biological test object could be found for semi-quantitative assay of the amount of activator in blood, studies were conducted on intestinal segments suspended in Ringer's solution. It is with these data that the present communication is concerned.

METHOD. Rabbits were killed by a blow on the neck, a piece of intestine removed 15 cm. from the pylorus, and after gentle cleaning away of intestinal contents, rings 2.5 cm. long cut. These were suspended in baths of oxygenated Ringer's solution[1] at 37°C. The baths were equipped with automatic temperature regulators and were of 40 cc. capacity. The renin employed was prepared by the method of Helmer and Page (1939). Plasma was prepared from heparinized blood and used within an hour after the blood was drawn. In several experiments no difference was observed in fresh plasma and plasma which had stood 24 hours in the refrigerator.

The experiments were conducted as follows. After the intestinal segment was contracting rhythmically and steadily, 1 or 2 cc. samples of

[1] Composition of Ringer's solution:

	grams per liter
Sodium chloride	9.0
Potassium chloride	0.417
Calcium chloride	0.24
Magnesium chloride	0.062
Sodium bicarbonate	4.0
Dextrose	8.0

plasma were added to the bath to insure that the plasma itself contained no constrictor substance. The segment was then washed twice with Ringer's solution and 2 cc. of renin added and allowed to remain for 5 minutes. After washing the segment three times, 2 cc. of plasma were again added and the constrictor action recorded. Without further treatment with renin, plasma was repeatedly tested until it was certain that the response was relatively constant. Segments of the same intestine were tested simultaneously with normal and hypertensive blood in the two baths of the apparatus.

In a few experiments, angiotonin prepared by the method of Page and Helmer (1940) was used.

Reaction of intestine to various constituents of the renin-angiotonin vasopressor system. Renin-activator prepared by fractional precipitation of
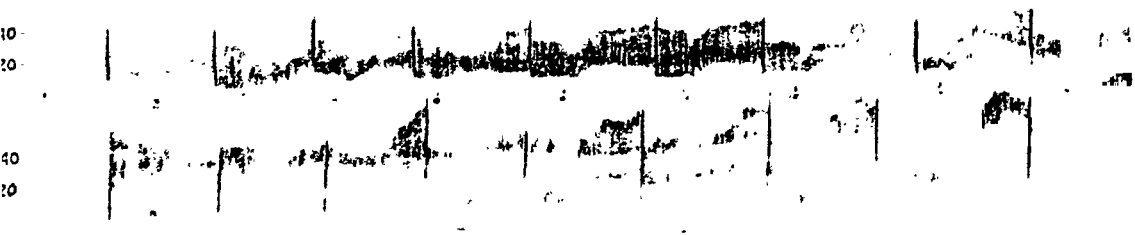
Fig. 1. Contractor action of plasma from normo- and hypertensive patients on isolated rabbit's intestine after treatment with renin. Number 1, 3 plasma (2 cc.) from patient with normal arterial pressure (114/70 mm. Hg); 2, 4 plasma (2 cc.) from hypertensive patient (188/118 mm. Hg); 5, 6 renin (2 cc.) in contact with intestine for 7 minutes; 7, 9, 11, 13, 15, 17, 19 addition of normal plasma; 8, 10, 12, 14, 16, 18 addition of hypertensive plasma.

blood with potassium phosphate when added to the bath in amounts of 1 to 2 cc. caused no contraction of the intestine. Neither ultra-filtrates of renin or of renin-activator were active either alone or mixed. Boiled renin plus boiled renin-activator was also ineffective. But if renin (0.1 cc.) and renin-activator (1.7 cc.) were mixed and added, immediate contraction occurred. The quantitative aspects of this reaction have been dealt with more fully by Page (1939).

Plasma (2 cc.) usually causes no contraction and renin (2 cc.) may or may not do so. Since plasma contains renin-activator, mixing renin and plasma yields a contractor substance. The contractor substance is doubtless angiotonin for angiotonin itself causes marked and sharp contraction of the intestine when added to the bath. Furthermore, repeated doses of angiotonin sensitize the intestine markedly, but treatment of the intestine with angiotonin does not cause it to respond when plasma is added subsequently.

These reactions have been employed as a basis for roughly estimating the amount of renin-activator in plasma. Renin is added to the tissue and then the excess washed away. When plasma containing activator is added, angiotonin is formed which causes the intestine to contract. It

TABLE 1

Contraction of intestine as a result of treatment with renin followed by plasma from normal and hypertensive persons

SUBJECT	B.P.	CONTRACTION IN MILLIMETERS OF INTESTINE AFTER ADDING SUCCESSIVE DOSES OF PLASMA						
		1	2	3	4	5	6	
Human hypertension								
	mm. Hg							
Normal...........	126/90	2	0	0	0	0	0	1 cc. plasma
Hypertensive.....	188/100	15	14	15	15	17	20	
Normal..........	120/70	0	0	0	0	0		
Hypertensive.....	170/90	20	9	8	10	12		
Normal..........	128/68	0	2	2	4	2	2	
Hypertensive.....	200/136	0	6	9	12	9	8	
Normal..........	120/70	1	2	1	2	1		
Hypertensive.....	198/118	36	9	13	14	20		
Normal..........	130/80	4	0	0	0	0	0	
Hypertensive	200/136	14	8	2	13	6	26	
Normal..........	115/70	4	4	6	5	13	12	2 cc. plasma
Hypertensive.....	198/118	12	19	19	27	30	38	
Normal..........	124/84	5	5	3	6	5	6	
Hypertensive.....	210/140	11	20	23	26	24	23	
Experimental hypertension								
	MEAN B.P.							
Normal..........	128	3	0	3	0	2	3	1 cc. plasma
Hypertensive.....	187	16	15	18	20	19	23	
Normal..........	130	0	0	0	0	0		
Hypertensive.....	180	7	3	10	10	10		
Normal..........	135	4	4	3	3	5		3 cc. plasma
Hypertensive.....	190	28	20	19	21	32		
Normal..........	130	5	5	3				
Hypertensive.....	186	12	10	14				
Normal..........	122	0	8	8	5			
Hypertensive.....	170	1	14	13	12			

probably also sensitizes it to further angiotonin formation. Hence, when the first plasma is washed away and a second, third or fourth dose of plasma is added, without re-treatment of the tissue with renin, contraction occurs.

Assay of plasma of normal and hypertensive persons and dogs. The bloods assayed were drawn from patients with typical essential hypertension and

from dogs with hypertension produced by the perinephritis method of Page (1939).

In plasma of both hypertensive persons and dogs, there appears to be greater activating power for renin than in normal blood (table 1).

DISCUSSION. Intestinal segments react with contraction to many substances or to changes in the conditions of the experiment. It is for this reason important to keep the conditions constant and to demonstrate that the reaction being tested is a specific one. These conditions have, we believe, been fulfilled in these experiments.

One other circumstance must, however, be kept in mind in interpreting these results. Page and Helmer (1940) were able to demonstrate the occurrence of an inhibitor to renin and angiotonin in blood of normal animals. It is therefore possible that instead of renin-activator being increased in hypertension, the amount of inhibitor is reduced. At present there is no way to decide between the alternatives, or the third one, that both may occur. Since nephrectomy appears to abolish one chief source of inhibitor, it is possible that lack of inhibitor causes increased activating power of hypertensive plasma.

It is probable that, under the experimental conditions described, renin is adsorbed by the tissue and, when renin-activator is added as contained in plasma, interaction occurs with liberation of angiotonin. Angiotonin causes the intestine to contract. Being water-soluble and easily diffusible, it is dissipated when the tissue is washed but enough renin is retained in the tissue to react with the next dose of renin-activator. Since angiotonin sensitizes the tissue, it is able to respond to smaller amounts of it. Addition of angiotonin itself is unable to produce the phenomenon because it is washed away with each washing of the tissue.

Plasma from certain hypertensive persons and from dogs with cellophane perinephritis and hypertension exhibits greater than normal power to activate renin. This adds one more bit of evidence suggesting that the humoral mechanism responsible for essential hypertension in human beings is similar to that in dogs with experimental hypertension. It also confirms, by an entirely different method, Kohlstaedt, Page and Helmer's (1940) observation that the renin-activating power of plasma is increased in experimental hypertension.

CONCLUSIONS

1. The reaction between renin and renin-activator is a specific one and the product of this reaction—angiotonin—causes strong contraction of isolated intestinal segments. This reaction has been employed to ascertain the renin-activating power of plasma.

2. Heparinized plasma derived from blood of some patients with essential hypertension causes greater renin-activation than does normal human

blood.   Plasma from dogs with experimental hypertension also exhibits this heightened power compared with plasma of normal dogs.   This suggests that the humoral mechanism in the two types of hypertension have much in common and that the hypertensive either has increased amounts of renin-activator in the blood, or decreased amounts of renin-inhibitor, or both.

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THE EFFECTS OF HYPERTHERMIA AND HYPOTHERMIA ON CERVICAL LYMPH FLOW[1]

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The experiments on the effects of local heat and cold upon cervical lymph flow (McCarrell, 1939–1940) suggested a further study of lymph formation and movement as a result of general heating and cooling of the body. The recent therapeutic use of hyperthermia and hypothermia has stimulated interest in the physiological changes, particularly those involving the circulatory system, brought about by abnormally high or low body temperatures. That alterations in lymph flow probably occur as a result of these circulatory changes has often been inferred, but actual experimental proof is meager. Ōtsuka (1936) reports an increase in thoracic duct flow in dogs after the injection of typhoid vaccine or "thermin." The highest temperature recorded was 41.1°C., and the lymph flow reached its maximum and fell off before the height of the fever was attained. No literature has been found on the subject of lymph flow during hypothermia.

The present study deals with the changes in lymph flow and protein content that occur in anesthetized dogs and cats subjected to high or to low environmental temperatures under such conditions as to cause profound body temperature alterations. The lowest body temperature obtained was 25°C., the highest 45°C. The experiments were acute, and thus no attempt has been made to determine the effects of prolonged exposure to extreme environmental temperatures.

METHOD. The experiments were carried out in an air-conditioned room in which temperature and humidity could be controlled at will. The animals were anesthetized by an intraperitoneal injection of nembutal (40 mgm. per kgm.). The right and left cervical lymphatics and the thoracic duct were cannulated at the base of the neck. The snout of the animal was attached by rubber bands to three metal uprights at the head of the animal board and by a double length of twine to a motor driven crank. As the crank revolved, the head was slowly flexed and extended, and thus a constant flow of cervical lymph was produced. The lymph, removed

from the cannulae during definite periods of time (10 or 15 min.), was collected in weighed tubes. The tubes were reweighed and the lymph flow calculated in grams per minute (thoracic duct) or in milligrams per minute (cervical). Protein percentage was determined refractometrically in all cervical lymph samples and in a few thoracic duct samples. The fat content of the latter was generally so high that accurate refractometric readings were impossible. The details of the above procedures have been described previously (McCarrell, 1939).

Before beginning lymph collections, the animal was given 300 to 500 cc. of warm saline solution by stomach tube to assure an adequate supply of water. Body temperature was recorded by a mercury thermometer, the bulb being inserted into the abdominal cavity through a small slit in the abdominal wall and secured with a purse string suture. In the majority of the experiments, subcutaneous temperatures of the head were obtained with a mercury thermometer sewed in place under the skin of the left cheek. Venous pressure was measured at intervals in the right external jugular vein by means of a hypodermic needle attached to a saline-filled manometer. Mean arterial blood pressure was recorded from the femoral artery with a mercury manometer, and pulse pressure with a membrane manometer. An intravenous injection of 1 cc. of a 1 per cent solution of curare was given at the beginning of each experiment to prevent alterations in lymph flow due to panting or shivering. Artificial respiration was administered through a tracheal cannula.

Each animal was prepared and a preliminary control period carried out at a room temperature of 25 to 28°C. For the heat experiments, the room temperature was then raised as quickly as possible to 40 or 45°C., 30 to 35 minutes being necessary to accomplish this. The high external temperature was maintained for periods ranging from 1½ to 3½ hours, depending upon the type of experiment. In some cases the room temperature was reduced to the control level as soon as significant changes in lymph flow were observed; in others, the heating was continued and the body temperature allowed to rise until circulatory collapse developed and the animal died. The average relative humidity of the room air was 11 per cent.

For the cold experiments the room temperature was reduced to about 10°C. by drawing cold outside air through the ventilating system. The animals were subjected to this cool environment for periods ranging from 2 to 3½ hours. Curare prevented shivering and the body temperature, which decreased gradually, reached minimum values of 25.6 to 29.7°C. before the experiments were terminated.

Nine dogs and two cats were studied under conditions of hyperthermia, and three dogs during hypothermia.

RESULTS. 1. *Heat.* In these artificially respired animals, panting was abolished by curare and the mouth was tightly closed with the twine

attaching the snout to the revolving crank. Thus the evaporation of moisture from the surfaces of the mouth and respiratory passages—the principal method of heat dissipation in dogs and cats—was reduced to a minimum. Under these conditions the body temperature began to rise soon after the room temperature was increased. The rise continued as long as the environmental temperature remained high, and, unless checked, led to circulatory collapse and the death of the animal.

The control period values for thoracic duct lymph flow in dogs ranged from 0.198 to 1.486 grams per minute; in one cat it was 0.134 gram per

TABLE 1

Cervical lymph flow and protein per cent during progressive hyperthermia

NUMBER OF ANIMAL	CONTROL PERIOD			FIRST CRITICAL TEMPERATURE			SECOND CRITICAL TEMPERATURE		
	Temperature	Average lymph flow	Protein	Temperature	Average lymph flow	Protein	Temperature	Maximum lymph flow	Protein
	°C.	mgm./min.	per cent	°C.	mgm./min.	per cent	°C.	mgm./min.	per cent
				Dogs					
1	37.0	28.3	3.46	41.1	50.2	2.86			
2	35.0	13.8	4.97	39.1	61.3	2.89			
3	38.5	16.8	5.22	39.9	72.8	4.78			
4	38.0	29.6	2.56	40.5	42.7	2.02	42.0	95.8	1.57
5	35.8	21.5	5.23	38.3	39.1	4.82	41.9	325.0	2.97
6	36.9	54.7	4.56	38.8	84.3	4.03	43.3	252.5	2.32
7	37.8	15.1	3.58	38.5	37.0	2.97	42.8	79.9	1.92
8	36.8	8.1	4.01	38.6	15.8	3.82	42.4	148.0	2.43
9	36.8	4.8	3.85	38.4	13.2	3.42	43.0	Saline injection before maximum flow reached	
				Cats					
10	37.0	23.8	4.02	None			43.4	60.7	2.75
11	37.0	3.9	4.18	40.0	14.2	3.58	43.5	30.9	2.04

minute. The flow tended to remain constant throughout the heating period up to the time of collapse, although in some experiments wide fluctuations occurred. A change was sometimes noted just before the death of the animal, but the direction of the change was not consistent. No general statement can be made as to the effect of hyperthermia on thoracic duct flow or protein content.

The cervical lymph flow, on the other hand, showed clear-cut changes at two separate critical levels of body temperature. Table 1 summarizes results obtained from the individual experiments. As the body tempera-

ture increased, cervical lymph production remained the same or less than the control period values until the first critical temperature zone was reached. At this point the flow began to increase, and attained values that were 1.4 to 4.5 times the original amounts. This increase was first noticed in dogs at body temperatures ranging from 38.3 to 41.1°C. Subcutaneous temperatures of the head were 0.3 to 0.6°C. higher than the corresponding temperatures in the abdominal cavity. The protein percentage decreased slightly as the lymph flow became more abundant. Reducing the body temperature restored the lymph flow to control values. One of the two cats failed to show a first critical temperature, the lymph flow remaining at the control level until collapse occurred. The second cat reacted similarly to the dogs, with a definite increase in cervical flow at 39.8°C.

If the body temperature continued to rise, the cervical flow was maintained at this higher level until a second critical temperature zone was reached at 41.9 to 43.5°C. At this point a tremendous increase in the cervical flow occurred, with ultimate values 3 to 18 times the control amounts being obtained. This augmented flow was characterized by a marked decrease in the percentage of lymph protein, the percentage at the height of the flow being approximately one-half that of the control period. The lymph flow fell off sharply just before death. The latter occurred in both dogs and cats when the body temperature reached 45.3 to 45.7°C.

Certain circulatory changes were brought about by the rising body temperature. An initial temporary decrease of mean arterial blood pressure occurred in some of the animals at the onset of the heating period, and was in most cases accompanied by a transitory decrease in pulse pressure. As the heating period continued, the mean arterial pressure and the pulse pressure in the majority of the animals showed gradual increases. At the time of the first lymph flow increase, the arterial pressure rise amounted to 6 to 35 mm. of mercury; that of the pulse pressure, 4 to 23 mm. of mercury. The arterial pressure in one dog failed to change, and in another dog exhibited a gradual decrease which continued throughout the experiment. The pulse pressure increased in all the dogs, decreased in one cat, and showed no change in the other cat. No correlation could be found between the relative amount of cervical lymph production and the relative pressure changes. No significant alterations of venous pressure were evident during this early period.

The circulatory changes occurring at the time of the second critical temperature were spectacular and quite uniform throughout the entire experimental series. The mean arterial pressure, which had remained the same or increased slightly above the level attained at the first lymph flow increase, exhibited a marked progressive fall at the time of the second tremendously augmented flow. The pulse pressure, likewise, began to

decrease at this time. The venous pressure increased 2.2 to 5.4 cm. of salt solution above the previous levels and then fell off rapidly as the circulatory collapse, which eventually caused the death of the animal, progressed.
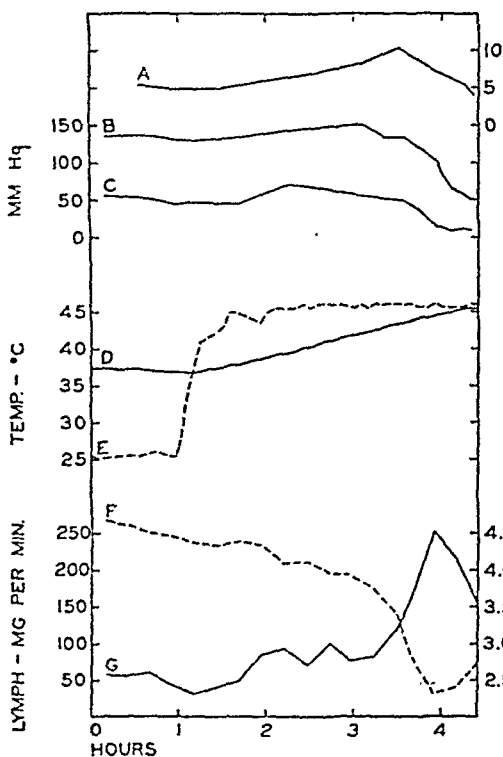
Fig. 1   Fig. 2

Fig. 1. Circulatory and cervical lymph data during progressive hyperthermia. *A*, venous pressure in centimeters of saline; *B*, mean arterial blood pressure in millimeters of mercury; *C*, pulse pressure in millimeters of mercury; *D*, body temperature in degrees Centigrade; *E*, room temperature in degrees Centigrade; *F*, cervical lymph protein per cent; *G*, cervical lymph flow in milligrams per minute. First critical temperature 38.8°C.; second critical temperature 43.3°C. See text.
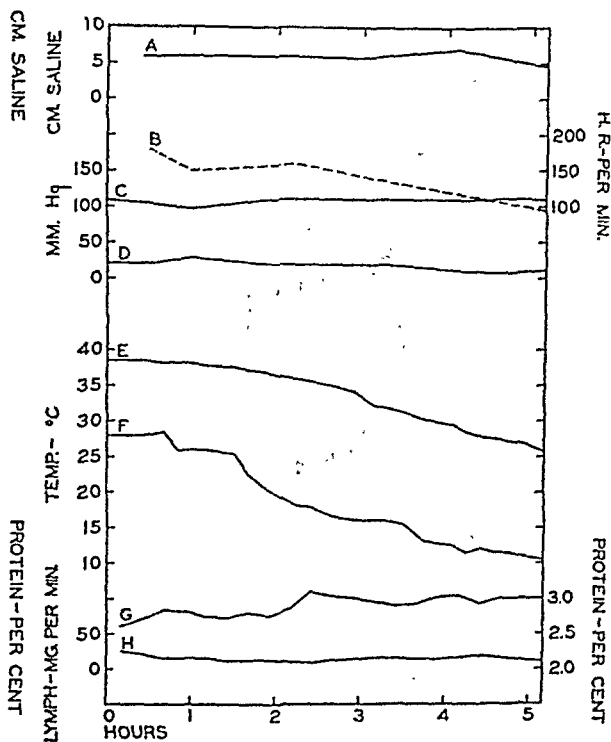
Fig. 2. Circulatory and cervical lymph data during progressive hypothermia. *A*, venous pressure in centimeters of saline; *B*, heart rate per minute; *C*, mean arterial blood pressure in millimeters of mercury; *D*, pulse pressure in millimeters of mercury; *E*, body temperature in degrees Centigrade; *F*, room temperature in degrees Centigrade; *G*, cervical lymph protein per cent; *H*, cervical lymph flow in milligrams per minute.

Figure 1, from data obtained during experiment number 6, shows the typical circulatory and cervical lymph changes which took place as the body temperature was gradually raised to a lethal level.

2. *Cold.* No striking changes were noticed in the cervical lymph flow as the body temperature decreased from approximately 38°C. to a minimum of 25.6°C. Subcutaneous temperatures throughout the cooling

period were 1 to 3 degrees lower than the corresponding abdominal cavity temperatures. There was a tendency for the flow to become slightly less and the protein percentage slightly higher than the control period values, but this situation is not uncommon when lymph is collected over a period of several hours. No conclusions could be drawn with regard to thoracic duct flow.

The circulatory manifestations, with the exception of the heart rate, were surprisingly inconspicuous, considering the magnitude of the body temperature change. The heart rate began to decrease at a body temperature of 35 to 36°C. and the rate became progressively slower as the hypothermia continued. Mean arterial pressure, pulse pressure, and venous pressure exhibited no consistent or significant alteration. Figure 2 represents the results of one typical experiment.

DISCUSSION. It is generally agreed that dilatation of peripheral blood vessels is a fundamental physiological reaction to an increased environmental temperature. Bazett (1938a) lists the factors which may cause this dilatation as: 1, the direct effect of heat on the vessels; 2, a reflex inhibition of vasomotor tone due to stimulation of surface receptors, and 3, inhibition of vasomotor tone through a temperature rise in the hypothalamic region. Vasoconstriction, with absorption of fluid in the splanchnic area, in addition to contraction of the spleen are compensatory mechanisms brought into action at the same time in an attempt to maintain an adequate blood supply to essential organs (Bazett, 1938b).

The rise in cervical lymph flow occurring at the first critical temperature (38.3 to 41.1°C.) I believe is due to an accelerated rate of capillary filtration. This is brought about, first, by arteriolar dilatation which increases the capillary pressure, and secondly, by capillary dilatation which enlarges the area available for filtration. Landis (1934) reports these as normal responses in a heated area. This shift of capillary fluid which leads to a greater flow of more dilute lymph, often at a body temperature only slightly above normal, may explain the heat edema sometimes noticed in the tropics and may be a factor in the common summer experience of swollen hands and feet.

The circulatory collapse exhibited in the present experiments was similar to that described for dogs subjected to excessive moist heat (Hartman and Major, 1935) and for humans who have collapsed, sometimes fatally, during or after hyperpyrexia treatments (Kopp and Soloman, 1937). Kopp and Soloman consider this condition an example of a typical shock syndrome. There is such marked dilatation of capillaries, arterioles, and veins that the normal compensatory mechanisms are overshadowed and an extremely serious condition develops. An inadequate venous return leading to stasis and anoxemia increases capillary permeability and causes the loss of an abnormal amount of fluid from the blood stream. Kopp and Solo-

man state that collapse occurred in their human cases at a body temperature of 106°F. (41.1°C.) or above. This is somewhat lower than the minimum collapse temperature for dogs and cats. However, circulatory distress in humans undergoing hyperthermia treatment can be augmented by uncompensated fluid loss through skin and lungs. Gibson and Kopp (1938) find this loss may range from 6 to 32 per cent of the plasma volume. Reduction of blood volume in this manner was not an important factor in the experiments under discussion. Collapse in these dogs and cats was caused by a reduction in the effective blood volume due, first, to the loss of fluid with the increase in capillary permeability, and secondly, to the uncompensated vascular dilatation.

In two instances, attempts were made to relieve the symptoms of circulatory collapse by reducing the body temperature and at the same time administering large amounts of fluid intravenously. In one experiment the body temperature had reached 43°C., arterial pressure had dropped to 85 mm. of mercury, and cervical lymph flow was showing a characteristic increase. The room temperature was then reduced to 25°C., and with the aid of wet cloths and a fan the body temperature was lowered to 38°C. During this time, 400 cc. of Ringer's solution were given intravenously. The experiment was terminated too soon to determine lasting effects, but a fair circulatory recovery was obtained and the animal seemed in good condition. In a similar experiment with a higher body temperature (44.8°C.) and a lower arterial pressure (65 mm. Hg) recovery was incomplete even with the intravenous injection of 600 cc. of a 6 per cent acacia solution. This illustrates the rather narrow margins concerned when dealing with a condition as serious as heat shock.

The tremendously increased lymph flow which appeared in the early stages of collapse must have been due, first, to temporary venous pressure rise which would tend to increase capillary pressure, and secondly, to an increased capillary permeability caused by stasis and anoxemia. The volume of lymph was so great that a protein percentage increase, usually indicative of definite capillary damage, was not apparent.

The findings of Yannett and Darrow (1938) indicate a further possible source of fluid which might eventually contribute to lymph production. They found that a shift of water from brain cells to extracellular fluid occurred in cats with rectal temperatures 4 to 6°C. above normal. There were no significant changes in liver or muscle and no determinations were done on subcutaneous tissue.

The first reaction to exposure to cold is vasoconstriction of peripheral vessels. This reduces blood flow and, therefore, the loss of heat from the surface of the body. If the skin temperature becomes low enough (0 to 10°C.), capillary dilatation and hyperemia, as described by Lewis (1927), may occur. Apparently, capillary conditions, even at a minimum body

temperature of 25°C., are not sufficiently altered to cause changes in the amount of capillary fluid transudation that can be detected in the cervical lymph flow.

Hamilton and Barbour (1925) placed dogs on blocks of ice, and report that the muscle and subcutaneous tissues of the cooled side had a greater water content than the uncooled side. Landis (1934) remarks that the difference may have been due to gravity. No temperatures were given, and it is possible that the skin temperature may have become sufficiently low to cause capillary dilatation and hyperemia. Hamilton, Dresbach and Hamilton (1937) subjected rats and kittens to severe hypothermia, and, with the exception of a slowed heart rate and loss of spontaneous movements, found no marked physiological changes at body temperatures as low as 75°F. (23.9°C.). At 65°F. (18.3°C.) or less, they report a marked subcutaneous edema with profound disturbances of the nervous, vascular, and respiratory systems. The edema shown by these animals was undoubtedly an expression of the circulatory disintegration that occurs as a lethal temperature is approached. Jackson and Alonge (1934) found that 62 per cent of a large group of rabbits exposed to extreme cold died when the body temperature fell to 19°C.

In the new hypothermia treatment for cancer (Smith and Fay, 1939), with rectal temperatures reduced to 85 or 90°F. (29 to 32°C.), it is probable that the lymphatic circulation may be reduced to some extent, but it is believed that any effects of the low body temperature in this respect are not pronounced.

SUMMARY

Nine dogs and two cats, anesthetized and curarized, were subjected to a high environmental temperature (40 to 45°C.) at an average relative humidity of 11 per cent. The hyperthermia thus produced caused marked circulatory changes culminating in circulatory collapse and death at 45.3 to 45.7°C. Cervical lymph flow increased and protein percentage decreased at two critical body temperature levels. The first rise, which amounted to 1.4 to 4.5 times the control values and which occurred at a body temperature of 38.3 to 41.1°C., was due to an increase in the rate of capillary filtration caused by peripheral hyperemia. The second increase in cervical lymph flow, amounting to 3 to 18 times the normal, appeared at a temperature of 41.9 to 43.5°C., was coincident with the beginning of circulatory collapse, and was caused by a tremendous increase in capillary filtration resulting from a high venous pressure and from capillary stasis and anoxemia leading to injury to the capillary endothelium.

Cervical lymph flow in three dogs subjected to low environmental temperatures (10°C. minimum) failed to be significantly altered when the body temperature was reduced to a minimum value of 25.6°C. With the

exception of a decreased heart rate, circulatory changes were not pronounced at this degree of hypothermia.

Thoracic duct flow tended to be so variable that no conclusions can be drawn as to the possible influence of changes in body temperature on its production.

The writer wishes to thank Dr. Cecil K. Drinker for suggesting this problem and for his advice during its execution. Miss Hope King's assistance is also much appreciated.

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THE FLOW, PRESSURE, AND COMPOSITION OF CARDIAC LYMPH

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THE LYMPHATIC SUPPLY OF THE HEART. In a recent anatomical paper, Patek (1939) has summarized the literature upon the cardiac lymphatic vessels, and then has added very thorough observations made by modern injection methods in the normally beating heart of the dog. Patek's findings were as follows:

1. There is a plexus of large and small lymphatic capillaries under the epicardium, the larger vessels lying practically upon the muscle and receiving afferents from the myocardial lymphatic plexus. The subepicardial vessels empty into drainage trunks which accompany the coronary blood vessels. *These drainage trunks eventually unite into a single trunk which drains the entire heart and leaves it on the anterior surface of the pulmonary artery.* Valves occur in the draining lymphatics, but are rare in the subepicardial lymphatic capillaries.

2. In the myocardium there is an extensive plexus of lymphatics, the smallest being about three times the diameter of the blood capillaries in the same region. There are no collecting trunks in the myocardium, and all the lymph in these vessels passes into the large subepicardial lymphatic capillaries on the outer surface of the heart muscle. The myocardial lymphatic plexus is very uniform in density from the subendocardial to the subepicardial vessels, and valves are rare.

3. The third plexus of lymphatic capillaries is subendocardial and drains into the myocardial plexus. Valves are uncommon.

In summary, lymph formed in any part of the beating heart passes to the subepicardial plexus and then into the valved draining trunks. The movements of the heart must force lymph in the three systems of lymphatic capillaries in various directions, but since they are all connected and since there is but a single way for lymph to leave the heart, there is apparently steady drainage into the large valved trunks which accompany the coronary vessels and unite into the final single cardiac lymphatic.

In our experiments, we have cannulated this lymphatic after it emerges

from the pericardium and before it enters a very constantly placed lymph node shown in figure 1. We believe that the lymph collected by means of this cannula represents the complete lymph flow from the heart and from no other structures. Our reasons for this belief are as follows:

a. Alterations in flow and composition of lymph taken from the cannula follow immediately upon changes in cardiac activity.

b. When dyes are injected in the heart, we have found them in this lymphatic alone. Furthermore, with a cannula in the cardiac lymphatic
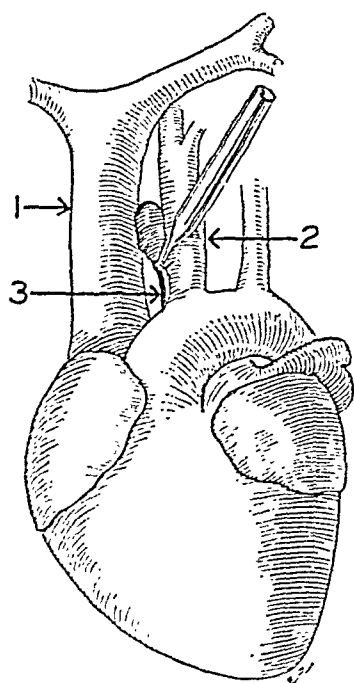
Fig. 1   Fig. 2

Fig. 1. Diagrammatic sketch of the anterior surface of the dog's heart and certain of the great vessels. *1,* superior vena cava; *2,* innominate artery; *3,* cardiac lymphatic cannulated just prior to entering the cardiac lymph node.

Fig. 2. Normal flow of cardiac lymph. *Ordinates,* milligrams of lymph per minute and arterial blood pressure; *abscissae,* time in hours.
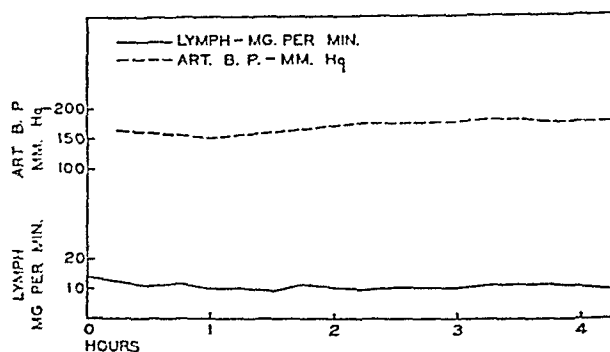
so that connection between this vessel and the thoracic duct is broken, no dye appears in either the thoracic or right lymphatic ducts after such injection into the heart muscle.

c. We have considered that lymph from the lungs might be mixed with the cardiac lymph, but have been unable to devise acute injection experiments which would prove this point. But on a single occasion an experimental dog showed pronounced pulmonary anthracosis, and lymph nodes at the root of the lung were quite black, being filled with pigment

carried to them by the lung lymphatics. On careful histological examination with the polarizing microscope, there were many fine unphagocytosed particles of crystalline silica in the pulmonary node and none in the cardiac node. It may be said that all particles were filtered out in the pulmonary node and that clear lymph from the lungs reached the heart node, but in the case of finely divided crystalline silica this does not happen. The material is not taken wholly effectually by phagocytes, and where two nodes are in series the second invariably receives a quota; and since these highly refractile particles are so easily recognized by the polarizing microscope, conclusions as to their absence are entirely safe and we believe this accidental finding, which must have followed a long period of dust inhalation, is important evidence that the cardiac lymphatic we have cannulated carries lymph from the heart alone.

EXPERIMENTAL TECHNIQUE. Dogs of above 10 kgm. weight are desirable, but in all work upon the lymphatics it soon becomes evident to the experimenter that the size of lymphatics and the degree of lymph flow are not wholly dependent upon size of animal. Anesthesia is accomplished by intravenous injection of nembutal, repeated in small amounts as required.

After tracheal cannulation and establishment of artificial respiration, the thorax is opened by removal of a section of the first three ribs about three or four centimeters from their sternal connections. The mammary arteries are then tied and the sternum sawed through just below the third rib, a block tie being passed around the lower end of the sternum to prevent venous oozing. This operation exposes the base of the heart and the great vessels. If any considerable amount of thymus tissue is present it should be removed, but usually the thymus does not extend as low as the site of cannulation of the cardiac lymphatic. This vessel is located by means of the cardiac lymph node, and its discovery will be greatly facilitated if bleeding into the loose tissues of the mediastinum has been thoroughly checked so that the operative field is clean.

In the beginning of our experience we found the vessel by the injection of a blue dye, T-1824, into the cardiac muscle, making a small hole in the pericardium and injecting into the muscle of the right ventricle by means of a fine needle. This injection will be necessary for any one who begins to look for this lymphatic, since it is embedded in fat and loose connective tissue, and, uninjected, is not easily seen save through the light of experience plus low-power binocular magnification. The vessel is usually about of the diameter of an ordinary pin. It is cleaned and tied, and then swells somewhat, which facilitates the incision for cannulation. The fine cannulae used are made of pyrex glass, 4 mm. in outside diameter and from neck to end are 7.5 cm. in length. The movements of the heart

and great vessels are always disturbing, but if the cardiac lymphatic is freed sufficiently cannulation can usually be accomplished, and the length of the cannula permits fastening it loosely in place by a stay suture in the left edge of the thoracic wound. The tip of the cannula in the cardiac lymphatic often rests upon the innominate artery and moves continuously during the several hours of the experiment. It has been a surprise to us that in spite of this, neither breakage of the lymphatic nor even small leaks of lymph have occurred unless brought about by our own manipulations in the course of the experiment.

Finally, in order to prevent coagulation of the lymph, a fine wire with a loop at the lower end about 2 mm. in diameter is dipped in dry heparin and placed in the cannula. Lymph leaving the cardiac lymphatic slowly dissolves this heparin, and clotting, always a difficulty in such work, ceases to need consideration.

While the cannulation is in progress and in order to be sure the animal is thoroughly supplied with water, 20 cc. per kilogram of Ringer's solution are given intravenously. Following such an injection, for reasons we cannot explain, lymph flow is not always rapid, but failure of flow cannot be ascribed to a lack of water in the tissues. In all cases femoral arterial blood pressure has been taken, usually with both a membrane and a mercury manometer.

Pressure measurements in the cardiac lymphatic were made by attaching a glass tube to the cannula in the cardiac lymphatic. They are thus maximal end pressures and give no idea of the normal side pressure in the vessel.

Flow of lymph was determined by pipetting lymph from the cannula into weighed tubes, usually through 10 minute periods, and then weighing. Occasionally, with free lymph flow, sampling periods were less, and with low lymph flow greater. Lymph protein and blood protein concentrations were determined by means of a Zeiss dipping refractometer, with frequent checks through micro-Kjeldahl analyses. In analysing for albumin, globulin was precipitated with 22.5 per cent sodium sulphate, filtered off, and the nitrogen determined by micro-Kjeldahl analysis, a modified Pregl apparatus being used for distillation. Globulin was determined by difference. The colloid osmotic pressures were measured by means of the Hepp micro-osmometer as described by Peters and Saslow (1939). Chlorides were determined by the method of Manery, Danielson and Hastings (1938).

EXPERIMENTAL RESULTS. *The flow of cardiac lymph.* Figure 2 illustrates an experiment showing the flow of cardiac lymph per minute and the arterial blood pressure over four hours' time. It is typical of many which show how uniformly the heart produces lymph while circulatory

conditions remain constant. The dog was a young adult, and the heart weighed 85 grams. In table 1 are found figures for the average flow of cardiac lymph taken from ten experiments which were technically entirely satisfactory. All these animals were young adults apparently in the best of health, and all had an intravenous injection of 20 cc. of Ringer's solution per kilogram of body weight prior to beginning lymph collections. The table permits no correlations between lymph flow and other possible factors such as body weight, heart weight, etc. It merely gives the average production of lymph from a single working organ profusely supplied with blood capillaries and lymphatics and under constant working conditions.

When the work of the heart is increased by intravenous injections of adrenin or ephedrine, lymph flow increases promptly and often to a sur-

TABLE 1

Average flow of lymph from the heart in ten dogs

NUMBER OF EXPERIMENT	WEIGHT OF ANIMAL	WEIGHT OF HEART	BLOOD PRESSURE	AVERAGE LYMPH FLOW PER MINUTE	AVERAGE LYMPH FLOW PER MINUTE PER GRAM HEART
	kgm.	*gm.*	*mm. Hg*	*mgm.*	*mgm.*
1	8.0	77.0	142	5.20	0.0675
2	11.8	111.0	129	7.72	0.0694
3	11.8	94.0	94	8.65	0.0920
4	11.8	91.0	130	9.38	0.1030
5	14.6	114.5	112	9.76	0.0852
6	11.0	85.0	160	10.47	0.1230
7	14.0	102.0	104	15.78	0.1547
8	14.4	91.0	120	15.81	0.1737
9	11.0	97.5	140	27.27	0.2786
10	11.5	91.5	112	27.56	0.3012

prising degree. Figure 3 is a chart of an experiment in which after one and one-half hours of normal conditions an intravenous injection of 130 mgm. of sodium nitrite was given. Blood pressure and lymph flow fell slowly. Toward the end of the third hour a dose of ephedrine (13.0 mgm.) was given intravenously. There was an immediate increase in cardiac activity, blood pressure, and lymph flow. In other experiments in which ephedrine was injected, the lymph flow remained high during the period of action of the drug. As may be anticipated, exactly similar results but more transient were obtained with adrenin.

Pressure in the cardiac lymphatic. We have measured the maximal pressure of the cardiac lymph in two animals by the simple expedient of attaching a narrow glass tube to the cannula in the cardiac lymphatic.

In one instance the pressure rose to 14.1 cm. of lymph. In the second, with a mean blood pressure of 112 mm. of mercury, it was 15.5 cm. of lymph, and as a result of an intravenous adrenin injection rose to 18.6 cm. of lymph.

The composition of cardiac lymph. The amounts of lymph which can be collected in experiments such as we have described are often too small to permit a reasonably complete series of quantitations. One of our principal interests has been the percentage of protein in the lymph. This has been measured in 18 experiments on different dogs, and frequently many times in the same experiment. Our lowest figure for cardiac lymph was 2.50 per cent, the highest 4.73 per cent, with an average of 3.69 per cent.
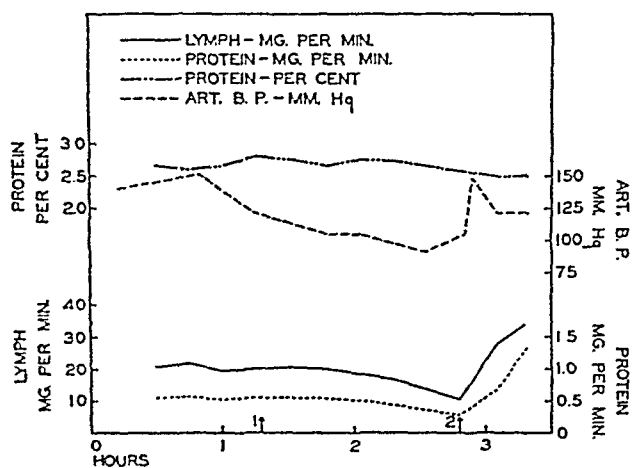
Fig. 3   Fig. 4

Fig. 3. Cardiac lymph flow, blood pressure, and per cent of lymph protein in a dog given sodium nitrite and ephedrine. *Ordinates,* lymph flow in milligrams per minute; *abscissae,* time in hours. At arrow *1* on abscissa, 130 mgm. sodium nitrite intravenously; at arrow *2,* 13 mgm. ephedrine sulphate.
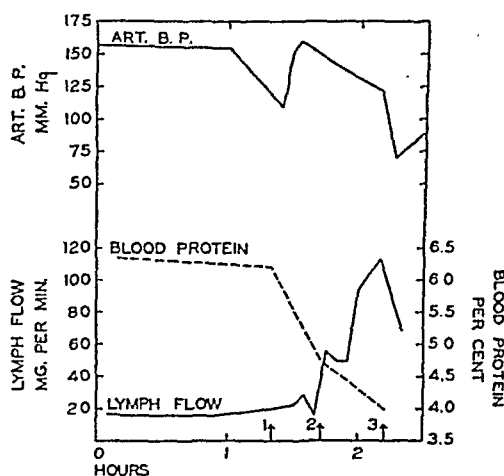
Fig. 4. Plasmapheresis and cardiac lymph flow. At the arrows *1, 2,* and *3,* blood was removed and washed red cells reinjected.

In table 2 we have listed experiments upon which we have been able to make a number of analyses, and of the animals in this table, nos. 5, 6, 7, and 10 appear in table 1, which deals with the amount of lymph flow per minute in these animals. The discussion of certain of the implications of the figures in table 2 is best reserved until the presentation of further data. It may, however, be pointed out that cardiac lymph, like lymph from all other parts of the body, contains the blood proteins, and clots; and that the pericardial fluid, which in addition to filtration through the walls of the blood capillaries, has also passed through a considerable fraction of the epicardium or pericardium, contains the blood proteins, and

clots, and is merely a slightly diluted lymph (Maurer, Warren and Drinker, 1940).

TABLE 2

A summary of certain factors in the composition of blood, cardiac lymph, and pericardial fluid in six dogs

NUMBER OF EXPERIMENT	TOTAL PROTEIN	ALBU- MIN	GLOB- ULIN	ALBUMIN / GLOBULIN	CHLO- RIDE	COLLOID OSMOTIC PRESSURE		Cl IN SERUM / Cl IN LYMPH AND PERICARDIAL FLUID
Blood serum								
	per cent	per cent	per cent		mgm./ 100 cc.	mm. H_2O	mm. H_2O per gram protein	
5	4.67	2.37	2.30	1.0		165	33.1	
6	6.31	3.56	2.75	1.3	418	239	37.8	
7	5.52	2.67	2.78	0.96	417	219	39.7	
10	5.36	3.50	1.86	1.9	412			
11	5.50	2.70	2.80	0.96		244	44.3	
12	8.31	3.06	5.25	0.58		302	36.3	
Lymph								
5	2.94	1.60	1.34	1.2		134	45.4	
6	3.60	2.26	1.34	1.7	475	169	46.9	0.88
7	3.15	1.86	1.29	1.4	455	142	45.0	0.92
10	3.91	2.60	1.31	1.9	421			0.98
11	4.70	2.30	2.40	0.96		189	40.2	
12	4.70	2.57	2.13	1.2		240	41.0	
Pericardial fluid								
5	1.12	0.58	0.54	1.1				
6								
7	1.84	1.18	0.66	1.8	438	72	39.1	0.95
10	2.66	1.12	1.54	0.7	447			0.92
11	1.20	0.75	0.45	1.7		56	46.0	
12	1.31				416	48		

TABLE 3

The appearance of horse serum after intravenous injection, determined by immunologic methods in cardiac lymph

	LYMPH + SALINE	ANTISERUM + SALINE	LYMPH				BLOOD	
			Control	Hours after injection			Hours after injection	
				1	2	3	2	3
Undiluted	—	—	—	+++	++++	+++++	++++	++++
1:50	—	—	—	+	++	+++	+++	++++
1:100	—	—	—	—	+	+	+++	+++
1:500	—	—	—	—	—	—	+	+

In order to demonstrate the permeability of the cardiac blood capillaries, we have cannulated the cardiac lymphatic and injected horse serum intravenously, and have determined the time of appearance of horse serum in the cardiac lymph.

Experiment 2. Weight of dog, 11.8 kgm.
9:00 a.m.   Nembutal anesthesia.
11:40 a.m.   Cardiac lymphatic cannulated.
1:40 p.m.   Normal lymph and blood samples collected; 50 cc. of horse serum given intravenously without observable effect on heart or blood pressure.
4:42³⁰ p.m.   Lymph and blood collections finished.

Table 3 summarizes the results and indicates that foreign protein readily enters the cardiac lymph from the blood just as it has been shown to do in the case of leg lymph, cervical lymph, and thoracic duct lymph after intravenous injection.

The same type of experiment has been carried out using gum acacia instead of horse serum.

Experiment 23. Weight of dog, 11.8 kgm.
9:00 a.m.   Nembutal anesthesia.
10:40 a.m.   Cardiac lymphatic cannulated.
1:10 p.m.   Control specimens of blood and lymph collected, and 3 grams gum acacia injected intravenously. Strength of solution 7½ per cent. No effect on heart and blood pressure.
4:20 p.m.   All specimens of blood and lymph collected.

Acacia in blood and lymph was determined as described by Maurer, Warren and Drinker (1940).

The results are shown in table 4.

The use of plasmapheresis and the Starling heart-lung preparation to illustrate certain features of lymph flow from the heart. The experiments so far discussed have been carried out upon animals which were in normal condition except for the anesthesia with nembutal, the open chest, and the cardiac lymphatic cannulation. The normal course of lymph flow has been altered by drug injections, but not by such radical procedures as are implicit in plasmapheresis and possible by use of the Starling heart.

Figure 4 illustrates a plasmapheresis experiment upon a dog weighing 11.5 kgm. After a long period of very steady conditions as to blood pressure and lymph flow, 650 cc. of blood were removed in two installments, as indicated by the arrows in figure 4, and the same amount of washed cells suspended in Ringer's solution was reinjected. The blood protein was reduced from 6.21 per cent to 3.99 per cent with a very great increase in lymph flow. Following a third and final blood removal and

reinjection of washed cells at 3:12 p.m., there was a serious fall in blood pressure and lymph flow—findings not infrequent in acute plasmapheresis when, after the experiment is pushed a certain distance, it seems as if the blood capillaries were damaged and for a time abnormally permeable. Before the first hemorrhage and reinjection (at 2:20 p.m.), blood protein was 6.21 per cent; preceding the second removal and reinjection (at 2:43

TABLE 4

The appearance of gum acacia in cardiac lymph after intravenous injection of 3 grams in a 7.5 per cent solution

	BLOOD	LYMPH
	mgm. per cubic centimeter	mgm. per cubic centimeter
Control.................................	0	0
After 1 hour..............................	3.0	0
After 2 hours.............................	2.83	0
After 3 hours.............................	2.63	0.26
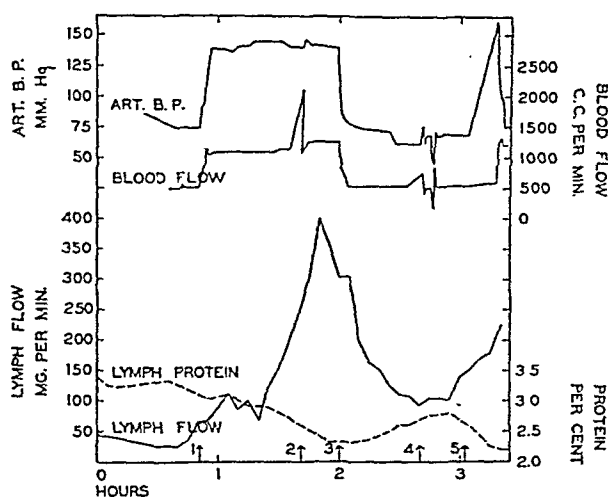
Fig. 5. Starling heart-lung experiment with cardiac lymph collection. At arrow *1*, cardiac inflow was increased and arterial resistance raised; at arrow *2*, 2.5 cc. of 1:50,000 adrenin were placed in the venous reservoir; at arrow *3*, cardiac inflow was decreased; at arrows *4* and *5*, 1000 cc. of Ringer's solution were added to the blood, reducing the blood protein from 4.68 per cent to 1.54 per cent.

p.m.), it was 4.74 per cent; and before the third removal and injection occurred (at 3:12 p.m.), it was 3.99 per cent. Serious fall in blood pressure did not take place until after the final hemorrhage, but the great increase in lymph flow shown in figure 4 takes place in the 29 preceding minutes when the blood protein was 3.99 per cent. The experiment so far as effect on lymph is concerned is entirely in accord with experiments on acute

plasmapheresis when lymph was collected from the thoracic duct and cervical lymphatics (Field and Drinker, 1931).

We have shown the increase in cardiac lymph flow which follows the augmented cardiac activity induced by ephedrine. That experiment and others in which adrenin was used do not give any satisfactory idea of lymph flow during normal increase in cardiac work, blood flow, and arterial pressure which result from severe muscular exercise. The single way to gain an idea of maximal production of cardiac lymph is through the use of a Starling heart-lung preparation in which it is possible at will to increase the inflow of blood to the heart, to increase peripheral resistance, and to add the effects of adrenin.

In collecting lymph from a Starling heart preparation, it is advisable to section the four upper ribs and remove the sternum from the upper end to below the fourth rib articulation. This gives more space for placing the necessary ligatures around the great vessels and makes it possible to tie the aorta without disturbing the lymphatic cannula, which should be in place prior to shifting from the normal to the heart-lung circulation. We have used the superior vena cava for blood inflow and the left subclavian artery for outflow. The mechanical arrangements of the peripheral circulation were of the usual type, and heparinized dog blood was the circulating medium. Figure 5 is a chart of a Starling heart experiment with cardiac lymph collection. The dog weighed 11.3 kgm., and the heart 91 grams. The combination of increased inflow of blood to the heart, increased blood pressure, and adrenin injection caused a lymph flow of 400 mgm. per minute. It is, however, doubtful if a flow above 300 mgm. a minute could be sustained under conditions simulating what would be the circulatory situation in a dog during a long run. The possibilities for the hound engaged in a 12 hours' chase are approximately 18 cc. of lymph per hour, or 216 cc. for the 12 hours. Taking a good-sized hound, such as the subject of our Starling heart experiment, with a heart weighing 91 grams, this means 2.4 cc. of lymph per gram of heart during the 12 hours.

DISCUSSION. The experiments which have been described seem to us to accomplish two things. First, to a limited extent, they describe a physiological mechanism in the heart which heretofore has had no attempts at measurement. What may be the significance of the lymphatic drainage of the heart in such conditions as rheumatic heart disease in children, when at autopsy the heart is so often edematous, or, if we may describe it more cautiously, seems to contain too much interstitial fluid, and what may be the significance of this system as advancing years bring on the fibrous changes in the heart called chronic myocarditis? These are problems in experimental medicine to which we can do no more than draw attention. Under normal circumstances the function of the lymphatics is obviously the same as in other parts of the body—namely, to

remove the protein-containing tissue fluid from the part, and after passage through a lymph node to return it to the circulation.

The second and to us more significant point in regard to these experiments is their bearing upon the function of the lymphatic system, and particularly the relation of lymph to tissue fluid. In brief, we have shown that in normal dogs cardiac lymph flows steadily and in small amounts. This lymph always contains protein—in 18 dogs measured under satisfactory conditions an average of 3.69 per cent, with 4.73 the highest percentage and 2.50 the lowest. These figures are comparable to protein concentration of lymph in other regions, notably, and where we have most figures, for lymph from the cervical lymphatics.

In 1931, two of us (Drinker and Field) on the basis of a number of collections of lymph taken from various parts of the body suggested "that capillaries practically universally leak protein; that this protein does not reënter the blood vessels unless delivered by the lymphatic system; that the filtrate from the blood capillaries to the tissue spaces contains water, salts and sugar in the concentrations found in blood, together with serum globulin, serum albumin, and fibrinogen in low concentration, lower probably than that of tissue fluid or lymph; that water and salts are reabsorbed by the blood vessels and the protein enters the lymphatics together with water and salts in the concentration existing in the tissue fluid at the moment of lymphatic entrance. The lymph from any given drainage area contains a varying amount of protein dependent upon the amount of water absorption which has taken place in the region from which the collection is made, and represents a cross section of the tissue fluid of the area in question." This suggestion has been criticized but never in terms of direct experiment—always through experiments in which the possible concentrations of protein in the tissue fluid have been calculated through observations upon the blood when a part has been subjected to a variety of experimental manipulations. There is no argument as to the identity of the salt content of lymph, tissue fluid, and blood. The single difficulty rests with the problem of the amount of protein in this fluid.

Since 1931, several direct experiments have appeared which bear upon the problem, and by direct we mean experiments in which tissue fluid and lymph may be compared by analysis, not by inference. First, Drinker, Field, Heim and Leigh (1934) measured the protein content of lymph and edema fluid in dogs whose legs had been made edematous by lymphatic obstruction. The results appear in table 5, and the identity of protein concentration between lymph and edema fluid needs no comment. Second, and in the same year, Weech, Goettsch and Reeves (1934), in dogs rendered edematous by plasmapheresis or by protein deprivation, showed a striking similarity in protein concentration of edema fluid and

lymph taken from the feet in a number of dogs. Table 6 illustrates their findings. Third, Maurer (1938) collected tissue fluid from frog muscle

TABLE 5

The protein content of lymph and edema fluid from dogs 1, 2, 3, and 4. Blood and lymph collected simultaneously

(Drinker, Field, Heim and Leigh, 1934)

ANIMAL	DATE	LEG	PROTEIN	
			Lymph	Edema fluid
			grams per 100 cc.	grams per 100 cc.
Dog 1	Feb. 25, 1933	Left	2.67	2.00
	Mar. 8, 1933	Right	2.45	2.20
Dog 2	Sept. 15, 1933	Left	3.37	3.37
	April 3, 1934	Left	2.48	3.45
Dog 3	Mar. 28, 1933	Left	2.28	2.75
	May 5, 1933	Left	2.97	3.17
	Oct. 16, 1933	Left	3.17	3.17
	April 6, 1934	Right	2.50	2.67
Dog 4	April 9, 1934	Left	2.55	1.86

TABLE 6

Comparative protein contents of edema fluid and the lymph collected immediately after cannulization

(Weech, Goettsch and Reeves, 1934)

DOG NUMBER	HIND LEGS				FORE LEGS				ASCITIC FLUID	NATURE OF EDEMA
	Right		Left		Right		Left			
	Edema fluid	Lymph	Edema fluid	Lymph	Edema fluid	Lymph	Edema fluid	Lymph		
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	
5	0.23	0.18								Nutritional
8-40	0.04		0.02	0.11					0.02	Nutritional
8-06	0.17	0.28	0.14	0.60		0.31		0.23	0.13	Nutritional
8-38	0.08	0.53		0.30		0.29			0.32	Nutritional
9-92						0.07		0.06	0.01	Plasmapheresis
6						0.32		0.15	0.03	Plasmapheresis
5-8	0.09	0.06	0.08							Plasmapheresis
9-1	0.86*	0.38	0.95*							Plasmapheresis
2-3			0.17	0.19	0.16	0.14				Nutritional
1-31	0.04	0.01	0.16*						0.01	Plasmapheresis

* These edema fluids contained blood. See text.

and found blood proteins invariably present. When his figures for the concentration of this tissue fluid protein are compared with the figures for protein concentration in frog lymph, there is again an identical result.

To these observations may be added those in this paper. Table 2 presents protein concentrations in the heart lymph of six dogs. The amounts of protein in the pericardial fluid of the same animals are also given, and while they are less than for the lymph, it still remains that they are far above the amounts of protein usually thought to exist in tissue fluid. Maurer, Warren and Drinker (1940) have given evidence for the belief that pericardial fluid is a simple extracellular fluid filtered from the blood capillaries. Since this fluid readily becomes bloody if the heart is manipulated, there is reason to feel that it comes from the heart, and is in fact a filtrate from the epicardial blood capillaries which passes not only through their endothelial walls, but also through the epicardial endothelium. The fact that the pericardial fluid contains the blood proteins in lower concentration than the lymph is, in our opinion, due to a double filtration—the concentration in the cardiac lymph representing the approximate concentration in the tissue fluid of the heart, and the pericardial fluid concentration representing that of the tissue fluid reduced slightly by a second filtration through the epicardium.

SUMMARY

1. A method for collecting the entire lymph flow from the heart is described.

2. Cardiac lymph flow varies directly with the vigor of the heart beat. It increases with dilution of the blood proteins and consequent enhancement of capillary filtration.

3. The composition of cardiac lymph is described in some detail in six dogs and is compared with that of the pericardial fluid.

4. The cardiac lymph is a filtrate from the blood capillaries. Normally it contains serum albumin and globulin and it clots. Furthermore, if horse serum is given intravenously, it can be detected immunologically in the lymph, and similarly gum acacia is also found in this lymph after intravenous injection.

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REACTIONS OF LARGE AND SMALL ARTERIES IN MAN TO VASOCONSTRICTOR STIMULI[1]

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It is commonly believed that the arterial circulation is controlled primarily through a flood-gate mechanism located principally in the arterioles and the small arteries supplying them. Larger arteries and main artery trunks have been considered as elastic reservoirs having little directly to do with vasomotor reactions.

The plethysmographic observations of Schretzenmayr (1) on the exposed median and large arteries of the animal established their synergistic participation in reactions in which the arterioles have been usually considered as dominant. The larger arteries were observed to react in the same direction as the arterioles in response to direct nerve stimulation, vasomotor reflexes, dilator and constrictor drugs. They were shown to be in a state of tone due to continuous vasomotor influence. Quantitative (but not qualitative) differences in the reactivity of large arteries showed regional differences but did not correspond with the classification into "muscular" and "elastic" arteries.

Encouraged by these data and the existence of selective reaction patterns in the skin (3), it seemed desirable to compare large and small artery behavior in man. The radial artery and finger pad were selected for several reasons: 1. This artery lies near enough to the surface to be conveniently recorded with the plethysmographic technique used. 2. The finger vessels are extremely active indicators of vasomotor reactions. 3. Previous study of the hand skin (3) showed uniformity in the small artery and arteriole responses over the entire hand, so that if differences occurred in the reactions of the finger pad vessels and of the radial artery, these differences would be more probably related to differences in size, and possibly innervation, than to topographical factors. 4. In addition, there seems to be general agreement (4) that the radial artery's vasomotor innervation is supplied in the same manner from the peripheral somatic nerves as is the case for the digital arteries, and that differences exist only in the abundance of the supply.

METHODS.   Finger vessel reactions were recorded with the photoelectric plethysmograph previously described (2).   When this plethysmograph is placed in contact with the skin over the radial artery, the volume pulses in the latter are recorded instead of skin vessel reactions, (the arterial blood supply to the skin is poor in this vicinity), since the effect of the radial pulse now dominates the amount of light returning to the photoelectric cell (2).   It seemed convenient to record the radial artery volume pulses on a constant base line.   This was done by employing a capacity coupled amplifier (to be described in a later paper) instead of the resistance coupled amplifier used in our plethysmographic studies.   The amplitude of the volume pulse so recorded was used as a criterion of the tone of the radial artery.   There is a good basis for this.   There is the common clinical experience that the amplitude of the oscillations of a large artery is proportional to the patency of its lumen, that the oscillations decrease in size when the artery is constricted by spasm or by organic obstruction.   It is realized, of course, that gross changes in the stroke of the heart, in arterial pressure levels, and also in the artery's distensibility with various pressure levels, will influence the amplitude of the volume pulse in the radial artery. But these influences either do not appear to contribute significantly to the experiments reported below or they are noted when they occur. Healthy male medical students served as subjects, in a semi-reclining position on a comfortable couch.   Room temperatures varied between 25.0°C. and 29.0°C. in different experiments but were usually constant within one degree during the experiment.

RESULTS.   Small waves, synchronous with respiration, are commonly seen in the radial volume pulse, and less frequently on the finger plethysmogram (fig. 1).   They are probably due to variations in the stroke of the heart with the respiratory cycle.

Spontaneous waves are a common occurrence in the small arteries of the hand skin.   They are considered as ordinarily having a vasomotor origin (3).   Their absence from the radial artery (fig. 1) seems to be typical but this is not always the case.   Increased amplitude in the radial volume pulse may occur at the time of the finger constriction (figs. 2 and 3).   We have been unable to decide whether this is a mechanical effect from the constriction of the finger vessels, (i.e., it is due to the rise in resistance peripheral to the radial artery increasing the systolic engorgement) without change in radial tone, or whether a relaxation of radial muscle has actually occurred (fig. 3).   Prolonged spontaneous waves in the fingers, probably of psychic origin, are apt to show also but to a less extent in the radial volume pulse (fig. 4).   In this case, increased radial tone seems to be the explanation since changes in heart action are probably negligible here, and since the changes in finger and radial volume pulses are in the same direction.   These more prolonged vasomotor discharges apparently

involve more vascular territory than the briefer ones, since the latter
superimposed on, or preceding or following the former, often do not
affect the radial volume pulse when the more prolonged discharges do.

Deep breath. The vasoconstrictor reflex in the digits from a deep breath
has been shown not to involve the skin areas of the head (3). The radial
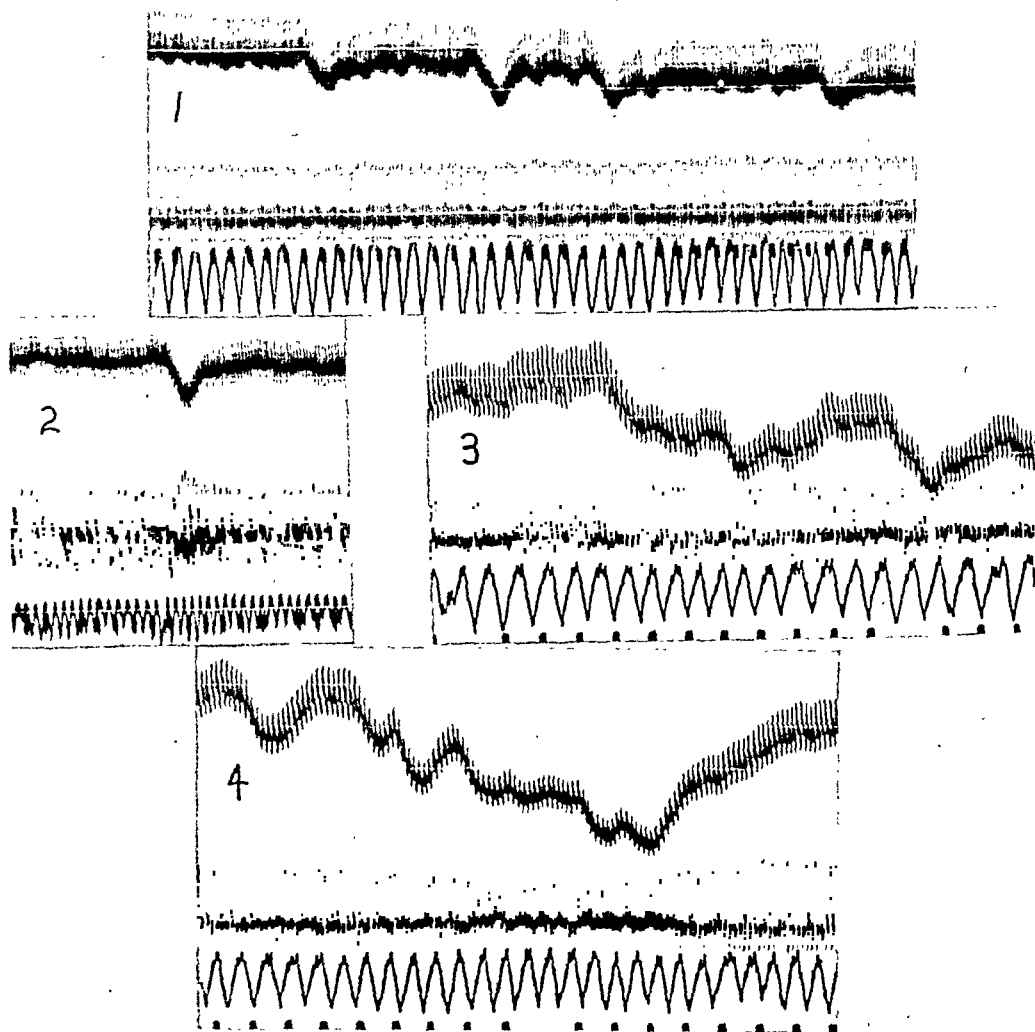
Figs. 1-4. Finger plethysmogram (upper record) and radial volume pulse (lower
record). Respiration. Time = 5 seconds. Spontaneous waves.

artery may also fail to participate (fig. 5). The initial decrease in ampli-
tude of the finger and radial volume pulses occurs simultaneously. That
this is probably not due to a constrictor reflex but rather to a temporary
decrease in left ventricular stroke is indicated by the increasing ampli-
tude of the radial volume pulse at the time that precipitate fall in finger
volume and volume pulse occurs. We conclude that the radial artery did
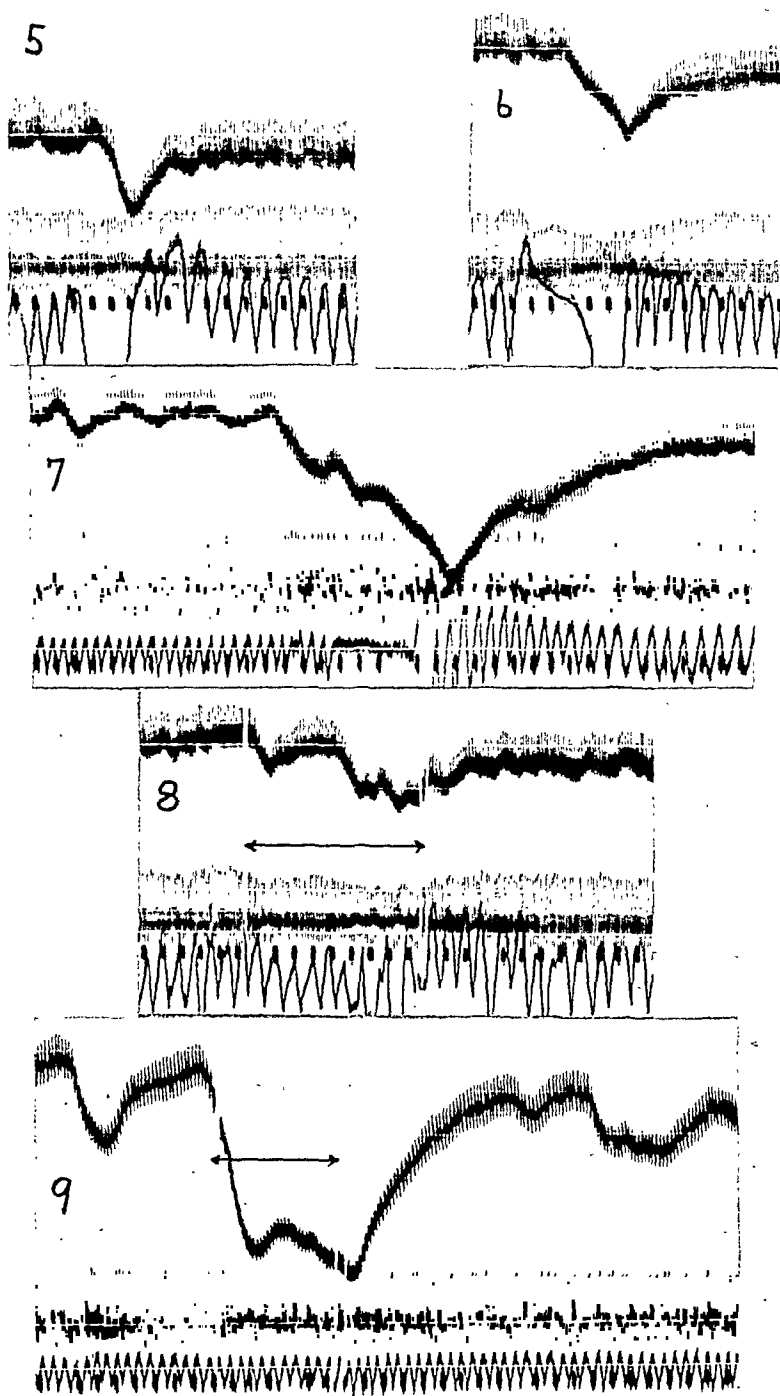
Figs. 5-9. Finger plethysmogram (upper record) and radial volume pulse (lower record). Respiration. Time = 5 seconds.

Fig. 5. Deep breath.

Figs. 6-7. Breath hold.

Figs. 8-9. Cold pressor test.

not share in the constrictor response in this instance. This seems to be the rule even in subjects who give intense responses to a deep breath in their digits.

Breath hold. This procedure has been offered as a convenient standard way of causing pressor responses (5). It elicits a more powerful and widespread vasoconstrictor discharge than does a deep breath if one may use the differences in the blood pressure responses as evidence. The breath hold causes a rise in arterial blood pressure varying in extent with the subject. Brachial blood pressure tends to fall or remain unchanged with a deep breath (6). One might expect, therefore, participation of the radial artery in the pressor response to the breath hold. Figure 6 illustrates such participation, but the constrictor effects are strikingly less than those in the finger. The initial decrease in the radial volume pulse is probably due to decreasing stroke of the heart. This shows likewise in the finger volume pulse. The beginning of vasoconstriction in the finger is timed with further progressive reduction in the radial volume pulse and the minima in both curves occur very nearly together. Recoveries likewise parallel each other in both records. Involvement of the radial artery in the constrictor response to the breath hold is, therefore, probable but it is obvious that the effects on the radial artery are relatively small. This difference in intensity of the response correlates qualitatively with differences in the richness of the vasoconstrictor innervation of the radial and digital arteries and arterioles and may well be due to such quantitative factors. That topography is an additional factor in the vasoconstrictor responses is suggested by failure of the head skin to participate (3). Likewise, failure of the radial artery to react is illustrated in figure 7 in which, despite profound constriction in the finger, the radial volume pulses are slightly increased during the finger constriction. Again, it is not possible to decide in this case whether the increased radial volume pulse is due to the rise in the resistance peripheral to the artery, or to changes in pressure levels, or actually represents decreased tone of the muscle of the artery. It would be interesting to know if the radial artery reactions are related to the extent of the rise in the arterial blood pressure in this test, but insufficient data have been obtained to attempt such a correlation.

Cold pressor test. The blood pressure responses of medical students seem to be somewhat higher in the case of the cold pressor test than in the case of the standard breath hold. This difference may be related to unrecognized small deviations from standard directions for the breath hold. The cold test presents a standard technique for presenting a painful stimulus. The vasoconstrictor responses are variable in intensity and topography as shown in the effects on blood pressure and on the circulation in various skin areas (3).

Three illustrations showing various degrees of response of the radial artery are provided in figures 8 to 10. The subjects of figures 8 and 9 gave

normal blood pressure responses.   In each of these, marked constriction of the finger arteries occurred without much participation of the radial artery.   In figure 8, the radial artery response parallels qualitatively that in the finger both during the constriction and also during the recovery from the procedure, but the changes in the radial artery are much smaller than those in the finger arteries.   In figure 9, the profound constriction in the finger is accompanied by a very slight constriction of the radial artery.   The spontaneous wave in the finger preceding the response to the cold did not involve the radial artery.   In figure 10, the response of an hyper-reactor with normal resting arterial blood pressure presents a striking contrast.   There was progressive constriction of the radial artery almost to obliteration.   Recovery was slow, requiring five minutes to return to the control level.   Yet, in this subject, the radial artery failed to share in the responses to psychic stimuli and in the spontaneous waves.
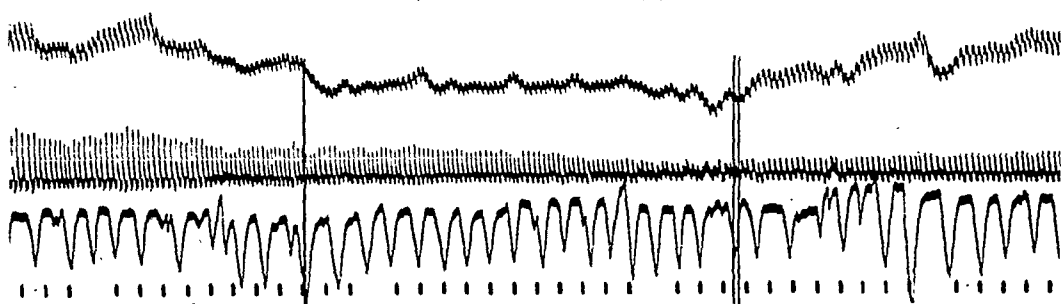
Fig. 10. Finger plethysmogram (upper record) and radial volume pulse (lower record).   Respiration.   Time: 5 seconds.   Cold pressor test between signals.

COMMENT.   We believe that we have shown that Schretzenmayr's thesis of synergistic participation of the large arteries in arteriolar and small artery reactions (1) is applicable only to special instances in the case of the radial-finger artery field in man.   As the stimulus eliciting a vasoconstrictor reflex increases in effectiveness, and as the response involves larger vascular areas, the radial artery may finally be involved, usually only to a moderate extent.

The extent of finger constriction does not seem to be a guide as to whether the radial artery will constrict or not.   Profound finger constrictions to loud noises, psychic stimuli, deep breaths are ordinarily unaccompanied by radial artery responses.   Breath holds which are effective in increasing the arterial blood pressure and in producing maximal finger constrictions have only a slight constrictor influence on the radial artery.   The cold test seems to be particularly effective in hyper-reactors, in effecting marked constriction of the radial artery, but it has only a moderate effect in those whose arterial blood pressure responses are normal, although the degree of finger constriction may be equally great in either type of subject.

It is possible that our method of observing the radial artery may have failed to reveal small changes in tone. Schretzenmayr's arteriograms (1) on the animal show marked quantitative but not qualitative differences in the changes in tone in the carotid and femoral arteries as compared to the large splanchnic arteries, the latter reacting much more vigorously. The reactions of the former were often overcome by the changes in blood pressure. However, his experiments differed radically from ours in that he employed powerful drugs and other procedures involving massive changes in the circulation. The two groups of experiments are not comparable nor are the two sets of data contradictory.

Our data support the possibility of selection with respect to the participation and the intensity of the participation in a given vasomotor response, of the large and small arteries in the same vascular field. They do not deny the synergistic action of large and small arteries in massive disturbances of the circulation. However, such action does not seem to be significantly involved in the more sharply localized and more moderate vasomotor reflexes studied in this paper.

SUMMARY

The possibility of synergistic participation of the large arteries in the reactions of the small arteries and arterioles supplied by them, has been studied in man, on the radial artery-digital artery field, using the photoelectric plethysmographs, previously described, to record the reactions.

The responses observed involved spontaneous waves (figs. 1–4), the reactions to a deep breath (fig. 5) to the breath hold (figs. 6 and 7), to the cold pressor test (figs. 8, 9 and 10), to loud noises and various psychic stimuli.

Radial artery participation in the vasoconstriction of the finger arteries was irregular and most obvious in instances of massive disturbances of the circulation. The degree of constriction of the finger arteries had little predictive value with respect to the occurrence of constriction in the radial artery. The data appear to show selection with respect to the participation, and with respect to the intensity of the participation, of the radial artery in the vasomotor responses of the small arteries and arterioles which it supplies.

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A STUDY OF ALLEGED QUANTITATIVE CRITERIA OF VASOMOTOR ACTION

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It is the vogue to utilize the magnitude of rise or fall in mean blood pressure induced reflexly or by humoral agents as a quantitative criterion for the degree of generalized vasoconstriction. Upon such criteria, far reaching conclusions are drawn regarding potency of hormonal agents and complicated central neural processes are inferred. This is done by eminent investigators despite the well known fact that it is not easy to evaluate the relative share that increased minute output, alterations in aortic capacity and elasticity and total peripheral resistance play in such pressor effects. Furthermore, the question whether absolute or percentile changes in blood pressure offer the better criterion of vasomotor action has never been settled (4, 5).

We have attempted to study these criteria of vasomotor power by comparing them with peripheral resistances calculated by a formula based on Poiseuille's law, viz., $R = \dfrac{P_m \times 1335}{V_t} = \dfrac{\text{dynes} \cdot \text{sec.}}{cm.^5}$, in which P_m denotes mean pressure and V_t, cardiac output per sec. For this purpose, mean carotid pressure was recorded by a properly damped Hg manometer and cardiac output was estimated by a calibrated cardiometer, carefully placed about the ventricles. Twelve dogs anesthetized with sodium barbital or amytal,[1] under mild artificial respiration, were used.

We may anticipate by stating that the investigation ended with the conclusion that the question at issue cannot be settled in this way because *peripheral resistance calculated in this way* is not solely determined by the algebraic sum of changes in size of the minute vessels. The evidence. leading to this conclusion is briefly reported in this communication.

Control values. In seven dogs with standard mean pressures of 80 mm. or more, the total resistance at the start ranged from 5880 to 9080 absolute units (A.U.). This is considerably higher than estimates in man, e.g., 1150 A.U. (O. Frank), 740 A.U. (Lauber), 520–1000 A.U. (Böger), 539

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[1] We are indebted to Eli Lilly and Co. for a supply of amytal for experimental purposes.

A.U. (Ranke), 1140–2740 A.U. (Böger and Wezler). (For references see Böger and Wezler (2)). The values are also higher than similar estimations on a few dogs by Böger (1) (4020–4470 A.U.) and by Broemser and Ranke (3) (2060–3100 A.U.); but much lower than estimates on rabbits by the same investigators (12,370–12,500 A.U.; 11,620–12,590 A.U.). In short, the calculated values seem to vary inversely as the size of the animal and not directly as one would expect.

In dogs, with open chests under artificial respiration, the values are not altered significantly by shock or shock-like conditions. Thus, in 3 dogs blood pressure had fallen to 46–50 mm. Hg at the start of observations, presumably due to operative shock. In these, the calculated peripheral resistance was 5249 to 7180 A.U., i.e., essentially that of dogs with normal mean pressures. This is contrary to much good evidence that peripheral resistance alters in this condition.

Reflex pressor reactions. In 22 tests, in which one or both central ends of vagus nerves were stimulated, mean pressures rose from the general average of 80 to 90 mm. Hg to ranges between 120 and 240 mm. Hg, depending on the strength of stimulation and number of nerve fibers excited. In every case, and regardless of coincident changes in cardiac output, the total calculated resistance increased with the rise in pressure. Not much more can be said. Since the initial mean pressures were so near 100 no essential differences in relation to the absolute or percentile increase would be expected, and none were found. However, when either the pressures or calculated resistances are plotted as a linear curve, the other does not follow linearly.

Since the dominant increase in resistance due to reflex pressor actions presumably occurs in the splanchnic area it seemed probable that at least equivalent increases in pressure and calculated resistance would result from occlusion of the thoracic aorta just above the diaphragm. This was never the case. Results from two illustrative experiments incorporated in table 1 show that the absolute and percentile increases in pressure following such occlusion are considerably less than is obtainable from reflex action and the percentile increase in calculated peripheral resistance is significantly smaller.

In twelve tests on animals, in which initial blood pressure ranged from 40 to 58 mm. Hg pressure, elevations of similar magnitudes were found possible. Thus, in one experiment pressures rose from 40 to 160 mm. Hg, a 300 per cent increase, and the calculated resistance rose from 2220 to 7170 A.U. Systolic output increased throughout the pressure rise. Correlation studies showed that throughout the rise, the actual elevation of mean pressure underrated the increase in resistance as calculated, but that the percentile increase overestimated it with respect to the calculated resistance.

. Similar but less pronounced effects were obtained in 12 tests in which both carotids were clamped and in nine tests in which the central end of a phrenic nerve was stimulated. The latter caused elevation of blood pressure by 22 to 30 mm. Hg in all except one, in which the rise was 38 mm. Hg. This was attended by 10 to 30 per cent increase in calculated resistance and offers evidence for existence of some pressor fibers in the phrenic nerves.

Effects of epinephrine and pitressin. A dose of 2 cc., 1:50,000 epinephrine solution was injected into vagotomized dogs in nine experiments. In general, calculated peripheral resistance again increased more in those experiments in which mean pressure rose most. The quantitative relation-

TABLE 1

INITIAL PRESSURE	MAXIMUM PRESSURE	PERCENTAGE INCREASE	INITIAL RESISTANCE	MAXIMUM RESISTANCE	PERCENTAGE INCREASE	EXPERIMENTAL PROCEDURE
Experiment 1—vagotomized dog—12 kilos						
mm. Hg	*mm. Hg*		*a.u.*	*a.u.*		
90	174	93	9,900	21,800	120	Central vagus stim.
80	130	62	14,400	25,400.	76	Occlusion aorta
94	140*	56	15,400	40,700*	164	Pitressin 1 unit
	160	70		24,800	61	
Experiment 2—vagotomized dog—8.5 kilos						
84	220	160	5,880	13,900	136	Central vagus stim.
86	234	172	6,570	14,960	127	
68	124	82	5,730	11,630	103	Occlusion aorta
74	154*		5,940	15,420*	161	Adrenalin 2 cc.
	182	146		13,180	121	1:50,000
46	156	· 240	6,800	23,100	240	Pitressin 1 unit

* Observations prior to those at maximal rise of pressure noted immediately below.

ships were not however as precise as appears at first glance. Thus, in experiment 2 (table 1) two previous tests with central vagus stimulation elevated mean pressures to 220 and 234 mm. respectively, the calculated resistances increasing from 5880 to 13,900 and from 6570 to 14,960 A.U. Following 2 cc. 1:50,000 epinephrine, the calculated resistance rose from 5940 to 15,420 A.U., but as pressure continued to rise it dropped again to 13,180 A.U. Since this was accompanied from the start by increase in heart rate and systolic discharge, i.e., by a considerable increase in minute output, one would anticipate a much greater elevation of mean pressure— both absolute and percentile—than during the central vagus stimulation. Actually mean pressure rose from 74 to only 182 mm. Hg and the percentile increase was less.

In four animals one "pressor unit" of pitressin, properly diluted, was administered intravenously. In every case the increase in calculated peripheral resistance was enormous but by no means always proportional to the elevation of pressure recorded. The data of table 1 illustrate two differing reactions: In experiment 2 a significant actual and percentile increase in pressure from previous shock levels took place and the calculated peripheral resistance likewise rose, perhaps fortuitously, by exactly the same percentile value as the mean pressure. In experiment 1, however, while both pressure and resistance increased up to mean pressures of 140 mm. Hg, the calculated peripheral resistance reached a maximum at this level and decreased significantly while mean pressure continued to rise. Comparison of changes in actual and percentile increase in mean blood pressure or actual and percentile increases in calculated peripheral resistance due to central vagus reflexes, aortic occlusion and pitressin are also shown in table 1. Anyone desiring an answer as to which of these three processes caused the greater vasoconstriction would be left in a quandary. These are only examples of numerous divergences of interpretation which would arise regarding quantitative changes in peripheral resistance induced by various drugs.

DISCUSSION. The original aim of this research was to test further whether absolute or percentile changes in mean pressure offer the better criterion of vasomotor action (4). To this end, coincident modification of blood pressure through changes in respiration and heart rate were largely avoided by double vagotomy and by ventilating the lungs of the animal so as to just abolish natural respiratory movements. It was hoped that secondary changes in systolic discharge would prove so slight that a comparison of changes in calculated resistance with absolute and relative changes in mean pressure would prove decisive.

It was found through study of cardiometer tracings that every significant change in arterial resistance due to vasomotor action causes *some* change in systolic discharge despite such controls. Generally this increases but, especially when the pressure elevation is abrupt and large, it may decrease. Again it may decrease at first, and increase later. Direct response of the myocardium by dilatation and increased venous return, in which contraction of the spleen plays a part, both seem to be concerned. These alterations in systolic discharge combined with the observations of Wiggers and Wégria (7) that the aorta decreases actively in size and virtually becomes more elastic are not without effect on mean pressure. It was recently shown by studies on artificial models (6) that considerable variation in the elasticity coefficient of an aortic system fortunately affects both mean pressure and calculated peripheral resistance to an insignificant extent only. In this conclusion we differ from Böger and Wezler (2) who believe it plays a dominant rôle in determining mean pressure. On the

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other hand, direct evidence obtainable on artificial circulation models (6) indicated that the relationship between systolic discharge and aortic capacity is a factor affecting mean pressure. Since mean pressure enters as a factor in the calculation of total resistance by the formula used it is apparent that these calculated resistances may also alter with changes in the ratio $\frac{\text{systolic discharge}}{\text{aortic capacity}}$, as well as with variations in size of minute vessels.

Such interpretations would explain many and perhaps all of our results, viz.: 1, that calculated total resistance varies inversely with the size of animals; 2, that it is not altered significantly during states of shock; 3, that it is not proportional to increase in mean pressure through agents which affect cardiac output, size of aorta, and peripheral arterioles to different degrees, and in different sequence, during the pressure rise; 4, that no correspondence exists between calculated resistances when pressures are elevated to equal levels by reflex action, hormones, etc., and 5, that the calculated resistance is less when the whole thoracic aorta is occluded than when its terminal minute vessels are constricted.

In conclusion, it becomes apparent that clarification of our notions regarding the meaning of "peripheral resistance" is needed. It is not clear at the present stage of progress whether the term should be advisedly restricted to frictional resistance occasioned through changes in size of small vessels and the viscosity of blood flowing through them or, following Böger and Wezler, whether central factors entering into formulae should also be included. If the latter, studies on artificial machines favor the view that this is not affected significantly by changes in aortic elasticity but rather by the variable relation between systolic discharge and aortic capacity.

In any event it becomes clear that the magnitude of peripheral vasomotor changes induced by nerves, hormones or chemicals cannot be assayed physiologically either by changes in mean blood pressure (actual or percentile) or by calculations of resistance in absolute units by formulae involving mean pressure as a factor.

SUMMARY

1. By use of simultaneous cardiometric records of the ventricles and mean arterial pressure in mildly ventilated, vagotomized dogs, data were obtained for calculating peripheral resistance changes according to the formula $R = \frac{\text{mean pressure}}{\text{cardiac output per sec.}}$ in $\frac{\text{dynes} \cdot \text{sec.}}{\text{cm.}^5}$, and comparing such values with the absolute and percentile changes in mean pressure.

2. Pressor effects on mean arterial pressure were induced in normal and "shock" dogs by reflex stimulation of the central vagus and phrenic

nerves, by bilateral carotid compression, by use of epinephrine and pitressin.

3. The results, supported by previous tests on artificial circulation machines strongly suggest that the quantitative estimation of changes in vasomotor tone of smaller vessels either by absolute or percentile changes in mean arterial pressures or by mathematical calculation of peripheral resistance is highly restricted even in experiments in which cardiac output is also measured. Mathematical calculations fail because mean arterial pressure, which enters into the formula, is affected by the relationship between systolic discharge and aortic capacity—but not, in our experience, by aortic elasticity. The formula does not take into account the aortic capacity in relation to systolic discharge either in the same animal or in animals of different sizes.

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THE RELATION OF TRANSPORTATION FORCE TO MOTILITY IN THE COLON OF THE DOG[1]

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It is common practice in the study of colon motility by the balloon technique to guy the system in place in order to prevent the shifting of the balloon to a lower level during the course of an experiment. Frequently, in the course of removing a balloon system at the close of an experiment, a very appreciable force is noted to oppose the withdrawal. This force, if allowed to act unopposed, would carry the balloon system to a lower level in the colon (1). In previous work we have observed the shifting of a balloon to the extent of several centimeters in the course of a 200 minute experiment (2). Such transportation is obviously the result of a force exerted by the colon. Previous work has revealed that transportation in the colon is intimately related to a certain portion of an active period. It seems reasonable then that the force exerted would be greatest during this portion of the period. On the other hand the force exerted against the balloon may be more evenly distributed than transportation within a given period, but without significant effect because of obstruction in the lower segment. To study the latter possibility, the force exerted in an attempt to move material in the lumen of the colon must be measured by as nearly an isometric technique as possible.

To record the force related to transportation efficiency, a technique was devised in which the carbon pile connected to a galvanometer was used to indicate the extent of force. This force, tending to transport contents of the colon to a lower segment was then correlated with motility. The single balloon technique previously described (2) was used to obtain records of motility. The carbon pile used for obtaining evidence of force involved in transportation was connected by a linen thread to the junction of the balloon and tube of the motility recording system (fig. 1). A glass syringe was attached to the galvanometer in such a way that the plunger could be moved simultaneously and to an equal extent with the indicated galvanometer deflection. With the syringe connected by way

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[1] The authors are indebted to Dr. A. J. Carlson, who made this study possible.

of a rubber tube to a water manometer it was possible to record even slight evidence of transportation force immediately beneath the motility tracing which indicated pressure changes in the balloon.

Data for correlating colonic motility with transportation force were obtained from five cecostomized dogs, which prior to this study had been trained to lie quietly for several hours at a time on a cushioned table. The balloon with its connections to a water manometer and carbon pile was inserted into the colon by way of the cecostomy to a depth of 10 cm.
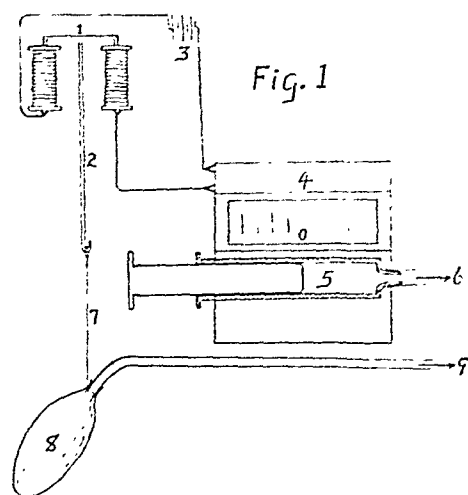
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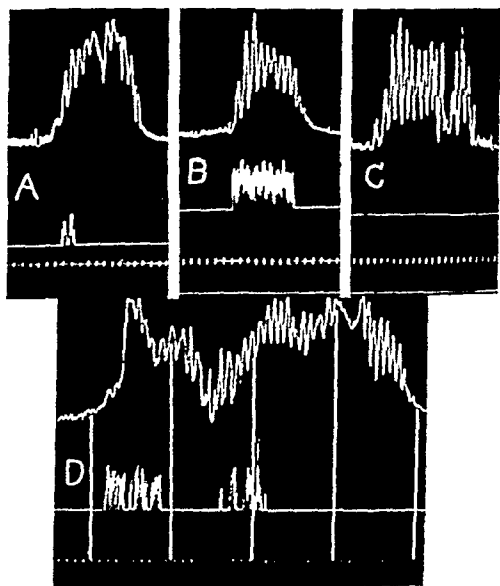
Fig. 2

Fig. 1. Apparatus used for recording colon motility and transportation force. 1 = carbon pile, 2 = arm of carbon pile, 3 = battery, 4 = galvanometer, 5 = syringe, 6 = syringe connection to water manometer, 7 = linen thread connection between arm of carbon pile and balloon, 8 = balloon, 9 = connection between balloon and manometer.

Fig. 2. Top line = balloon tracing of colon motility. Middle line = syringe record indicating galvanometer deflection as a result of transportation force. Bottom line = time in minutes.

beneath the skin. The linen thread connection between the carbon pile and the balloon was then made taut, and the balloon inflated according to a standard method (2) previously described.

Any force exerted upon the balloon tending to displace it to a lower level of the colon made more taut the linen thread connected to the arm of the carbon pile and gave as a result a deflection of the galvanometer. Each deflection was measured by means of the syringe previously described and recorded simultaneously with its occurrence. A tracing thus obtained recorded at each instance motility and its associated transportation force or "pull pattern." throughout the course of each experiment.

This method of obtaining a correlation between the transportation force and motility is more significant from a qualitative than a quantitative viewpoint. Qualitatively the exact relation could be followed between any force acting to displace the balloon and the type of motility recorded at that instant. Quantitatively, with a view to exact measurements on the extent to which the transporting force was acting was not practical with this apparatus, although estimates occasionally indicated as much as 200 grams of pull. Since, by this technique, the qualitative relation is more significant than the quantitative measurements, the term "pull pattern" may be more suitable than transportation force.

In a total of 25 experiments, each of 200 minutes' duration, a transportation force was observed only twice unassociated with activity. This may be explained as in a previous paper (2) by a consideration of force which may have been exerted upon the tube above the balloon either directly or indirectly through colon contents. A pull pattern was observed in 59 per cent of the 161 active periods recorded. These active periods

TABLE 1

The relationship between the average number of minutes of activity and transportation force per 50 minute period

	50 MINUTE PERIODS			
	1	2	3	4
Activity per 50 minutes....................	28	30	29	28
Transportation force per 50 minutes........	3.9	4.5	5.2	3.2

varied in duration from 1 to 104 minutes. Of the active periods 54 per cent were less than 12 minutes in duration, while 83 per cent were less than 24 minutes. These figures are in striking agreement with those previously reported (2).

A tabulation of the data (table 1) relative to the motility in each 50 minute period showed activity to be present 56 per cent of the time in the first period, 60 per cent in the second, 58 per cent in the third, and 56 per cent in the fourth period. The pull pattern was evident 8 per cent of the time in the first 50 minute period, 9 per cent in the second, 10 per cent in the third, and 6 per cent in the fourth period. From the data it is obvious that a pull pattern existed in a relatively small percentage of the time as compared to that occupied by activity.

Gross observation revealed a close relationship between the active period and the appearance of transportation force, while a tabulation of data shows a wide discrepancy between the duration of an active period and duration of transportation force. A more detailed analysis, therefore, was necessary to determine the more intimate relation of the pull pattern

to the active period.   This analysis was obtained by dividing each active period into four equal parts and tabulating the duration of transportation force in each quarter.   Thirty-three per cent of the total transportation force appeared in the first quarter of the active period.   This percentage was increased to 36 per cent during the second quarter and reduced to 19 per cent and 11 per cent respectively during the third and fourth quarters.   This particular observation is a partial confirmation of previous work (2), namely, that transportation during the first half of an active period (fig. 2, part A) is greater than during the second half.   The fact that the transportation force is as great in the second quarter as in the first, in contrast to our previous findings relative to transportation, is probably due to the technique employed which did not permit the balloon to descend.

Although the pull pattern is practically always related to an active period, the intensity of the active period is not an invariable index to
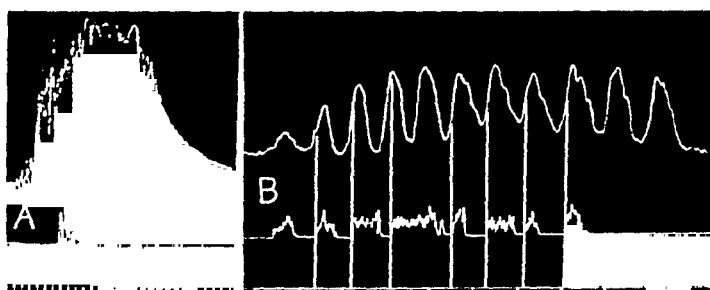
Fig. 3. Part A = Transportation force is correlated with the systolic phase of the type III contraction.

Part B = Rapid drum-showing that the start of the "pull pattern" occurs during the systolic phase of the type II contraction.

the transportation force.   Periods of activity of low intensity (fig. 2, part B) as indicated by pressure changes in the balloon are often associated with a pull pattern of considerable length.   On the other hand, active periods of as great an intensity (fig. 2, part C) may have no associated pull pattern.   During the course of an active period one or more type III contractions may appear.   Since the pull pattern seems to be related to the type III period (fig. 2, part D) such an active period which contained more than one type III contraction would tend to have the pull pattern spread more evenly throughout the period.   These facts are comparable to previous observations on transportation in which the balloon was free to move.

Since the pull pattern (fig. 3, part A) was most evident during the systolic phase of a type III contraction, the speed of the kymograph was increased in order to relate the transportation force to the different phases

of a single type II contraction. In the great majority of instances (fig. 3, part B) the beginning of a pull pattern was found to be definitely related to the systolic phase of a type II contraction.

The data obtained from 17 charts, each of 75 minutes' duration, revealed that 67 per cent of the pull patterns began during the systolic phase of type II contractions. Seventeen per cent started at the peak of type II contractions, while only 10 per cent began in the diastolic phase. Occasionally the tone of the colon was sufficiently high to practically obliterate the appearance of rhythmic contractions. Only 6 per cent of the total pull patterns started during this time. The low quantity of transportation force present during high colon tone is in keeping with the evidence presented by Quigley, Highstone and Ivy (3) that high intestinal tone is not conducive to the transportation of a bolus, and confirms our previous observation (2) on the transportation of a balloon in the colon.

SUMMARY

1. A technique is described by which a force acting to displace an object in the colon could be correlated with the motility at that instance.

2. Transportation force in the colon is intimately related to the active period.

3. The time during which transportation force is exerted upon an immovable object in the colon is greatest during the first half of an active period, and least during the last quarter.

4. The intensity of an active period is not necessarily indicative of the quantity of transportation force.

5. The systolic phase of type II and type III contractions is more efficient in exerting a transportation force than other phases.

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DECUSSATION OF THE SACRAL AUTONOMIC PATHWAYS OF THE BLADDER FROM THE HYPOTHALAMUS[1]

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During recent years, the brain stem and the spinal cord have been extensively explored in an attempt to locate the reactive regions for the contraction of the bladder (Kabat, Magoun and Ranson, 1936; Wang and Ranson, 1939a). Its efferent pathway has further been delimited by stimulating the anterior hypothalamus with lesions in the different regions of the brain stem and spinal cord (Wang and Ranson, 1939b). It was found that the response is generally not abolished if the lesion is limited to one side. Evidently, the sacral autonomic pathway of the bladder is partially crossed, but the location and extent of crossing are not clear (Gruber, 1933). The present investigation was undertaken to determine more accurately the level or levels of crossing.

METHODS. Chronic unilaterally and bilaterally hemisectioned cats were prepared. The operation was achieved through the dorsal approach under intravenous nembutal anesthesia (40 mgm./kgm.). Five levels were chosen: midbrain, medulla, upper cervical cord, lower cervical cord and lumbar cord. In the midbrain hemisections, the lesions were made in a plane extending from the inferior colliculus dorsally to the rostral part of the pons ventrally. The second hemisection at another level, if desired, was done about 2 to 3 weeks after the first operation. The animals were observed frequently for bladder and other visceral disturbances.

Experiments were performed on some 30 cats, three or more weeks after the final operation. At this time all the animals were in good physical condition. Light nembutal anesthesia was used (20 mgm./kgm., intravenously). A suprapubic incision was made to expose the urethra, into which a glass cannula connected with a tambour was inserted. Both hypogastric nerves were cut, the wound was then closed and 25 cc. of warm saline were injected into the bladder. Blood pressure was recorded from one of the carotid arteries by means of a mercury manometer.

The cat was then placed in the prone position, the skull trephined and bipolar bare-tipped nichrome wire electrode introduced into the hypo-

thalamus with the aid of the Horsley-Clarke instrument. The stimulating current was provided by a Harvard inductorium having a dry cell (1.5 V) in the primary and the secondary coil at 9 cm. The hypothalamus immediately caudal to the optic chiasma and at the level of the median eminence was explored millimeter by millimeter.

After the experiment was completed, the animal was perfused with 10 per cent formalin and the level of the lesion in the spinal cord determined. The hypothalamus and the spinal cord and/or the brain stem at the level of the hemisection were fixed in 10 per cent formalin, embedded in nitrocellulose and stained by the Weil method. The point of stimulation that yielded maximal responses and the extent of the hemisection were determined microscopically from a study of serial sections.

RESULTS. *Unilateral hemisection.* Animals with unilateral hemisections at the level of the midbrain, medulla or spinal cord showed little in the way of viscero-motor disturbances. For the first few days, there was usually a slight miosis and relaxation of the nictitating membrane on the side of the lesion, but all showed much improvement and after a month or so the differences in the size of the pupils and in the extent of relaxation of the nictitating membranes were almost imperceptible. All of these animals could micturate normally. Stimulation of either side of the hypothalamus yielded both pressor and bladder contractions in every case except cat 9 (see table 1). In cats 5 and 8 the hypothalamus on the normal side was stimulated again after section of all the sacral roots on that side. Good bladder responses were obtained (fig. 1).

Bilateral hemisections. With bilateral hemisections through the midbrain on one side and the upper cervical cord on the other, the animals behaved like the former group and could all urinate voluntarily. However, if the hemisections were placed at the levels of the upper and lower cervical cord, or of the cervical and lumbar cord, the animals were unable to micturate normally at will. "Autonomic bladder" with almost constant overflow would develop such as seen in animals with complete cord transections. For this reason, the urine was pressed out twice a day in order to prevent the formation of ulcers in the perineum. In addition, stimulation of the hypothalamus in this group of animals (cats 16–22, table 2) failed to yield bladder contractions although spontaneous rhythmic contractions in no way connected with the stimulation were often seen (fig. 2). On the other hand, stimulation of the hypothalamus in the animals with hemisection through the mesencephalon on one side and through the upper cervical cord on the other (cats 12–15, table 2) resulted in bladder contractions, and in two of them both halves of the hypothalamus were equally active.

Pressor responses were obtained in all animals with one of the hemisections placed in the midbrain or the lumbar cord. The hypothalamus

TABLE 1

Bladder and pressor responses following stimulation of the anterior hypothalamus in the chronic unilaterally hemisectioned cats

CAT NUMBER	LEVEL OF HEMISECTION	MAXIMUM CONTRACTION OF THE BLADDER		RISE OF BLOOD PRESSURE IN THE CORRESPONDING BLADDER REACTIVE REGION	
		Normal side	Hemisectioned side	Normal side	Hemisectioned side
		mm.*	mm.*	mm. Hg	mm. Hg
1	Midbrain	29	25	7	10
2	Midbrain	19	17	14	12
3	Midbrain	15	14	14	20
4	Medulla	21	18	23	20
5	C_2	25	23	56	56
6	C_2	25	25		
7	C_6	31	23	34	32
8	C_6	27	16	27	30
9	C_6	39	0	44	18
10	L_3	27	25	44	44
11	L_4	30	26	34	34

* The measurement refers to the height of the contraction of the bladder as indicated by the lever attached to the tambour and has no absolute quantitative meaning. In the individual experiment, however, the larger the value, the greater the force of the contraction of the bladder.
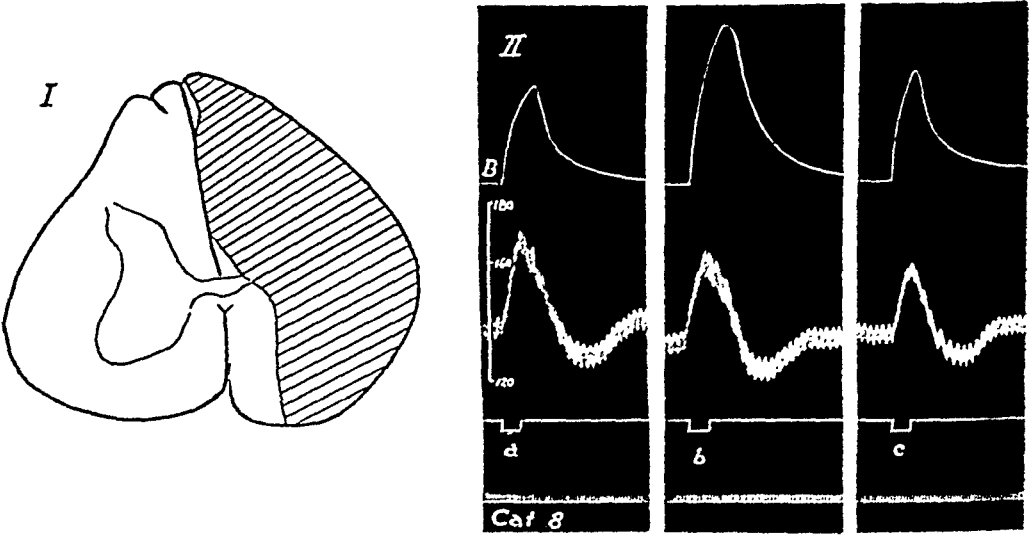
Fig. 1. I—Transverse section through the spinal cord of cat 8 at the level of C_6, the extent of lesion being shown by oblique lines.

II—Kymograph records of responses to hypothalamic stimulation in the same cat. Record a was taken from that of the hemisectioned side; b, from that of normal side, and c, from the same point as that from which b was taken after section of all the sacral roots of the ipsilateral side. Tracings from above downward represent bladder (B), blood pressure, the signal and time in seconds.

TABLE 2

Bladder and pressor responses following stimulation of the anterior hypothalamus in the chronic bilaterally hemisectioned cats

CAT NUMBER	LEVELS OF HEMISECTIONS	MAXIMUM CONTRACTION OF THE BLADDER		RISE OF BLOOD PRESSURE IN THE CORRESPONDING BLADDER REACTIVE REGION	
		Upper hemi-sectioned side	Lower hemi-sectioned side	Upper hemi-sectioned side	Lower hemi-sectioned side
		mm.	*mm.*	*mm.* Hg	*mm.* Hg
12	Midbrain-C_2	29	26	14	18
13	Midbrain-C_1	26	25	8	23
14	Midbrain-C_2	Trace?	35	20	24
15	Midbrain-C_1	0	33	?	10
16	C_2-C_6	0	0		
17	C_1-C_7	0	0	26*	28*
18	C_2-C_7	0	0	0	0
19	C_4-L_3	0	0	24	62
20	C_8-L_2	0	0	28	38
21	C_8-L_5	0	0	30	40
22	C_7-L_3	0	0	42	68

* The rise of blood pressure in this case was similar to that obtained on the spinal cats (Clark and Wang, 1939). They were delayed and sustained responses.
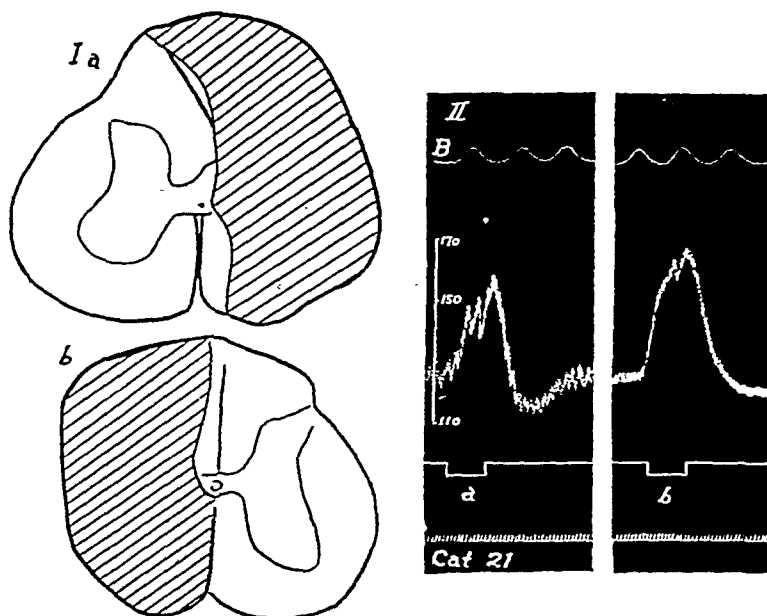
Fig. 2. I—Transverse sections through the spinal cord of cat 21 at the level of C_8 (above) and L_5 (below), the extent of lesions being shown by oblique lines.

II—Kymograph records of responses to hypothalamic stimulation in the same cat. Record *a* was taken from that of the cervical (C_8) hemisectioned side, and *b* from that of the lumbar (L_5) hemisectioned side. Tracings from above downward represent bladder (B), blood pressure, the signal and time in seconds. Note the spontaneous rhythmic contractions of the bladder, which were often seen in chronic transectioned cats.

in the group with the midbrain lesion (cats 12–15) was generally less re-
active, particularly in cat 15. In cats 17 and 18, the hemisections were
placed at the level of the upper and lower cervical cord. No prompt
pressor effect was obtained. Blood pressure was not taken in cat 6 (table
1) and cat 16 (table 2).

DISCUSSION. It is difficult to section exactly half of the spinal cord;
it is even more difficult to ascertain how much of the cord is functionally
intact, especially if the section is made acutely. For these and perhaps
other reasons, Spiegel and MacPherson (1925) could not decide whether
or not the bladder impulse crosses in the cervical and thoracic cord. How-
ever, we are now certain that the bladder impulse descends only in the
lateral white columns and not in the dorsal and ventral white columns
(Wang and Ranson, 1939b). Furthermore, in this experiment, narrow
strips bordering the median sulci of the spinal cord are definitely shown
to have no correlation with the descending bladder impulse (fig. 2). The
procedure of preparing chronic hemisection, on the other hand, would
certainly eliminate the acute effect of trauma (edema, etc.) on the intact
side. It appears to us also that severance of both hypogastric nerves
is indeed necessary, especially in case one of the hemisections is placed
in the lower lumbar cord. It is known that the hypogastric nerves formed
largely from the white rami of the second to fifth lumbar segments contain
some motor component to the bladder (Gruber, 1933), stimulation of
which may yield a small contraction of the bladder. Such responses
may be easily interpreted as the crossed component of the sacral autonomic
system.

Difficulty was encountered in maintaining cats with hemisection at the
level of the medulla. In the single experiment with section in the medulla,
a large portion of the medial reticular formation on the sectioned side was
found to have been left intact. Nevertheless, the case is included in table 1
because this structure does not carry any fibers mediating impulses to
the bladder (Wang and Ranson, 1939a). At the level of the midbrain,
the situation is quite different. Not infrequently, the contraction of
the bladder was produced by stimulating the region bordering the midline
(Kabat, Magoun and Ranson, 1936; Wang and Ranson, 1939a). For
this reason, a number of experiments had to be discarded for the lesion
was not extensive enough to exclude all the reactive region on the side of
the section. In the few experiments here reported, some midline struc-
tures have also been left uncut, but it is doubtful if they have any sig-
nificance for mediating the bladder impulse.

All the animals with unilateral hemisections with the exception of cat 9
showed decussations between the hypothalamus and the level of hemisec-
tion. The most rostral decussation is demonstrated in cats 1–3, that is,
a cross connection exists anterior to the midbrain lesion. In two cats

with cervical hemisections (cats 5 and 8), the bladder remained reactive to stimulation of the hypothalamus on the normal side after the sacral roots on its side were cut (fig. 1). There must therefore be another crossed connection caudal to the cervical lesion. The experiments with double hemisections at the levels of the upper and lower cervical cord or the cervical and lumbar cord, gave no evidence of any decussation at levels between C_2 and L_5. The lower decussation, then, is located caudal to the lumbar section. In the case of cats 12–15 with hemisection of the midbrain on one side and of the cervical cord on the other, a decussation at some level between the two lesions is demonstrated. We have no satisfactory explanation to offer in the case of cat 9 and of cat 15, in which no decussation is demonstrated rostral to the cervical cord and midbrain respectively. The points of stimulation are identified at the desired level and they are symmetrical; the lesion is also rightly placed. In a single experiment to show the effect of unilateral section of the cord on the contraction of the bladder induced by hypothalamic stimulation (Magoun, Ranson and Hetherington, 1938), the reaction was abolished to stimulation of the hypothalamus on the side of the lesion and was retained on the opposite side. There might be some variation in animals of the same species.

These multiple crossed connections at first seem superfluous. However, work reported elsewhere indicates that they exist. Kabat, Magoun and Ranson (1936) found that stimulation of the midline structures at the level of the diencephalon gives rise to good contractions of the bladder and suspected crossing in Meynert's and the supramammillary commissures. Barrington noted in 1925 that cats with bilateral lesions in the tegmental region of the pons ventral to the superior cerebellar peduncles showed permanent inability to empty the bladder, and therefore postulated the existence of a micturition center in that region. In the present series of experiments, the nature of the decussation at these levels is not clear; it may be a multiple one in the diencephalon and the pons, or a diffuse one in that region. As to the crossing in the lumbar region, numerous investigators have agreed that a reflex center chiefly concerned with micturition is located between the second and seventh lumbar segments (Gruber, 1933). And it is known that the centers there are connected with commissural fibers, and under ordinary circumstances the two halves act in harmony (Griffiths, 1895; Stewart, 1899). On the other hand, the absence of crossing in the entire spinal cord down to the upper lumbar segment is indeed very striking. Stewart (1899) who stimulated the upper cervical cord with a needle electrode found no response of the bladder if the cord is hemisectioned below the stimulated region on one side and in the thoracic cord on the other. Possibly because the cord was acutely hemisectioned, his negative findings have not apparently been

taken seriously. We have the advantage of not only using a chronic preparation but also stimulating a region which is more remote from the lesion. The fact that those cats cannot micturate voluntarily further substantiates our conclusion that the bladder impulses do not cross in the entire spinal cord down to the upper lumbar segment.

The pressor impulse descends from the hypothalamus and crosses partially in the brain stem and the spinal cord below the cervical region. This is in line with the evidence presented by Harrison, Wang and Berry (1939) on the unilaterally sympathectomized cats. In addition, our data indicate that there is no decussation of the pressor impulse in the cervical cord. And the delayed and sustained rise of the blood pressure in cat 17 is probably of a hormonal origin (Clark and Wang, 1939).

CONCLUSION

The anterior hypothalamus has been stimulated in chronic unilaterally and bilaterally hemisectioned cats for the contraction of the bladder. It is found that there is no decussation in the entire cervical, thoracic and upper lumbar cord. Cross connections are, however, located in the brain stem and the lower lumbar segments. The decussation in the former, that is, the brain stem, may be either multiple or diffuse in nature.

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FURTHER STUDY ON THE GASTRO-INTESTINAL MOTILITY FOLLOWING STIMULATION OF THE HYPOTHALAMUS[1]

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The numerous attempts to determine the effect of the hypothalamus on gastro-intestinal motility have led to conflicting and contradictory observations (Sheehan, 1940). By stimulating the hypothalamus in the unanesthetized cats, Kabat, Anson, Magoun and Ranson (1935) observed under fluoroscope immediate cessation of peristalsis and loss of tone of the stomach and small intestine. Beattie (1932a), Beattie and Sheehan (1934) and Heslop (1938b), using various methods such as naked-eye observation, x-ray and other recording means, found an excitatory effect on stimulation of the anterior hypothalamus and inhibition only limited to that of the posterior region. Masserman and Haertig (1938) on the other hand, reported that inhibition was obtained with a stronger current, while weak stimulus always yielded an excitatory response. The present study is conducted to supplement the previous observation made in this laboratory by exploring the entire hypothalamus to uncover its various effects on the stomach, intestine and colon, under a different set of conditions.

METHODS. With the aid of the Horsley-Clarke stereotaxic instrument, responses of the gastro-intestinal tract to faradic stimulation of the hypothalamus from the supraoptic region to the mammillary bodies were observed. In the first series of some 30 cats, light ether anesthesia was administered through a tracheal cannula. A midline abdominal incision was made and the abdominal muscles were tied around the neck of a shallow glass container. Suspension of the animal in the upright position was made by support of the head through the frame of the stereotaxic instrument and by strings passing under the supra-spinous ligament. Part of the small intestine and colon fell naturally into the container, in which warm Locke's solution was kept at 39 to 40°C. It was later found to be more convenient to observe the intestinal movements through an elliptical window made of transparent x-ray film with animal lying on

its back. The window and the abdominal wall were raised so that part of the gastro-intestinal tract could be seen.

At first animals were given about 100 cc. milk 3 hours before the acute experiment. In such a preparation, the gastro-intestinal tract usually showed occasional outbursts of activity, chiefly in the form of peristalsis. Difficulty was encountered in differentiating the spontaneous activity from that which resulted from stimulation. Inasmuch as a definite excitatory effect was often obtained during our early exploration, we confined our present study to its motor effect by choosing to use fasting animals.

In another series of 20 cats, chloralose was used instead of ether. A dose of 70 mgm. per kilo body weight, injected intravenously, was usually enough to keep the animal quiet. Balloons of various sizes were inserted into the stomach, small intestine and colon, and connected to water manometers for recording the tone and motility of each respective organ on a slowly moving kymograph. In all these experiments, the stimulus was given through a bipolar nichrome wire electrode connected to the secondary coil of a Harvard inductorium with a dry cell (1.5 V) at the primary. The distance between the primary and secondary coils varied from 9 to 12 cm. In 4 cats the spinal cord was sectioned at the upper thoracic segment after a positive response was demonstrated. Bilateral vagotomy was done in 9 others.

The anterior hypothalamus of 7 chronic cervical spinal cats was similarly explored. Kymograph records of the intestine and colon were taken in 4 of them and visual observations made in the rest.

The sites of stimulation in the hypothalamus were checked histologically with sections stained by Weil's method. The reactive points were plotted in 4 representative levels (figs. 1–4).

RESULTS. The intestine and colon of the fasting animals were generally quiet. There was no essential difference in the results obtained with light ether and chloralose anesthesia. When the anterior hypothalamus was stimulated with a weak but effective stimulus, usually for a period of 15 to 60 seconds, there appeared at first some definite blanching of the small intestine. The color returned immediately after the cessation of the stimulus; sometimes, it appeared more reddish than before. And a short while later, there not infrequently occurred swinging movements, sometimes segmentation and occasionally peristalsis. In most instances, the responses occurred in a few intestinal loops, and only in exceptional cases were the movements generalized. The colon appeared to be more responsive and peristaltic waves were more frequently observed (fig. 5). A second stimulation of the same region usually brought about an equal or a smaller response, depending upon the length of the interval between stimuli. Generally speaking, 10 to 15 minutes was found to be an adequate interval. When a response was obtained the second time, it usually
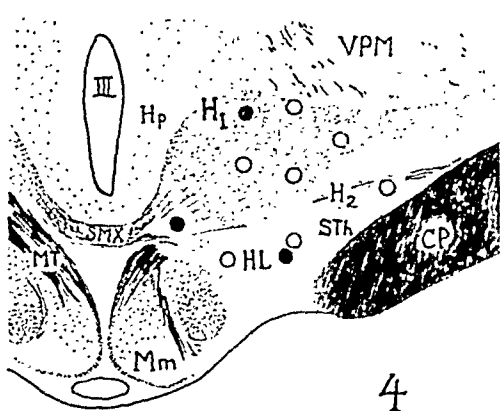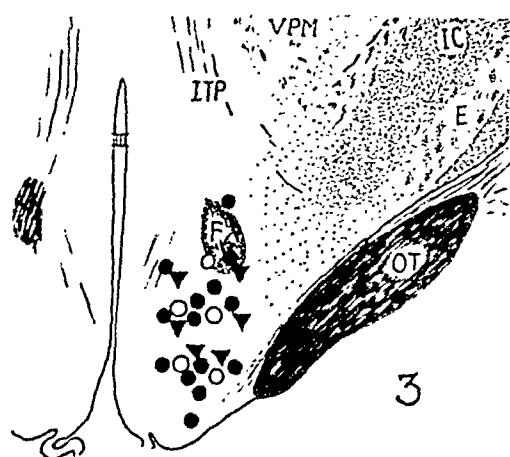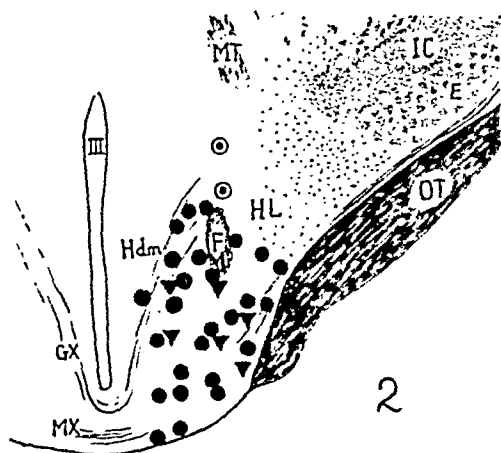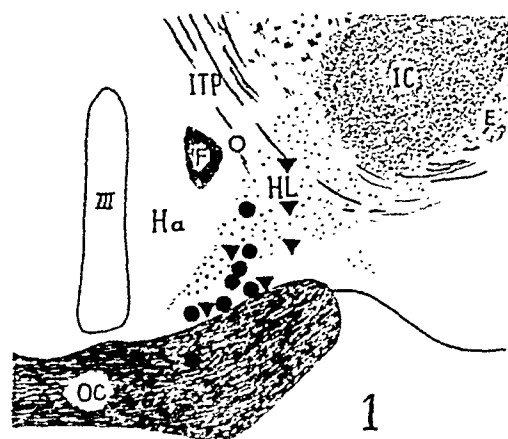
Fig. 1. Transverse section of the hypothalamus at the level of the optic chiasma. This figure includes the data from 7 cats in which various responses were obtained in this plane.

Fig. 2. Transverse section of the hypothalamus at the level of the supraoptic commissures. This figure includes the data from 13 cats in which various responses were obtained in this plane.

Fig. 3. Transverse section of the hypothalamus at the level of the median eminence. This figure includes the data from 11 cats in which various responses were obtained in this plane.

Fig. 4. Transverse section of the hypothalamus at the level of the mammillary bodies. This figure includes the data from 6 cats in which various responses were obtained in this plane.

Symbols and abbreviations for the figures 1–4

The four figures represent sections of the hypothalamus taken from four different cats. Solid circles (●) represent delayed excitations of the gastro-intestinal tract following unchanged activity during stimulation. Solid triangles (▼) represent delayed excitations following inhibitions during stimulation. Solid circles with circumscribed circles(◉) represent delayed excitation following immediate excitation during stimulation. Empty circles (○) represent excitation during stimulus. *CP*, cerebral peduncle; *E*, nucleus entopeduncularis; *F*, fornix; *IC*, internal capsule; *ITP*, inferior thalamic peduncle; *GX*, Ganser's commissure; H_1, H_1 field of Forel; H_2, H_2 field of Forel; *Ha*, anterior hypothalamic area; *Hdm*, nucleus hypothalamicus dorsomedialis; *HL*, nucleus hypothalamicus lateralis; *Hp*, nucleus hypothalamicus posterior; *Mm*, nucleus mammillaris medialis; *MT*, mammillothalamic tract; *MX*, Meynert's commissure; *OC*, optic chiasma; *OT*, optic tract; *SMX*, supramammillary decussation; *STh*, subthalamicus nucleus of Luys; *VPM*, nucleus ventralis posteromedialis; *III*, third ventricle.

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occurred in the same few loops of intestine with the same latent period and the response lasted for approximately the same length of time. If the electrode was moved 1 mm. upward or downward, the intensity and duration of the response might vary, but the latency appeared to be fairly constant. In general, the latent period was 40 to 60 seconds (average, 52). It depended somewhat upon the length of stimulus; the longer the stimulus, the longer the latent period. In most cases, the excitatory responses would not appear before the stimulus was terminated. On the other hand, there were cases in which the excitatory response appeared in the midst of a prolonged stimulus and the latent period was not much lengthened. The response, the increase of tone and movement as repre-
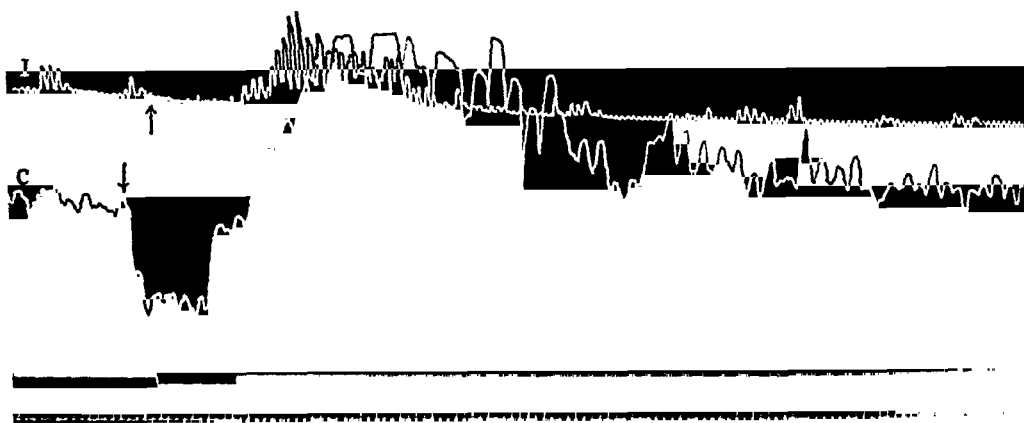
Fig. 5. Kymograph tracings showing the delayed excitatory responses of the small intestine (I) and colon (C) with loss of colonic tone during stimulus. Arrows indicate the commencement of stimulus. Time in 6 seconds. Small excursions of the tracing (I) are respiratory.

sented in figure 5, was always gradual in onset. It might take a minute or two to reach maximum and vanished so slowly and smoothly that sometimes it was difficult to make out the total duration of response. On the whole, it could be said that it lasted for 2 to 7 minutes, or longer in a few exceptional cases. Bilateral vagotomy in 6 of these cats was found not to abolish this type of delayed excitatory response (fig. 6).

As the intestine and colon were quiet under the control condition, it was often difficult to observe any inhibition. In a few cases in which there was spontaneous activity, stimulation did not invariably obliterate the regular peristaltic waves. In such cases, stronger stimulus did not alter the response, except perhaps the initial blanching was more marked. On the other hand, there were cases with inhibition of tone and other activities during stimulus, but almost always superseded later by increased tone

and contractions (fig. 5).   Uncomplicated inhibition was only occasionally observed.

On account of its anatomical location, movement of the stomach was not constantly observed through the window.   In 15 cats, a balloon was inserted into the stomach through the esophagus.   In many cases, the change during and following stimulus was small and complicated by the vigorous respiratory movements.   In 2 cats in which the response of the small intestine and colon was very marked, the stomach showed also a similar delayed excitatory response.   The effect in these 2 casts was sustained for more than 15 minutes.

When the hypothalamus at the level of the infundibulum and mammillary bodies was stimulated, sometimes an entirely different type of response was
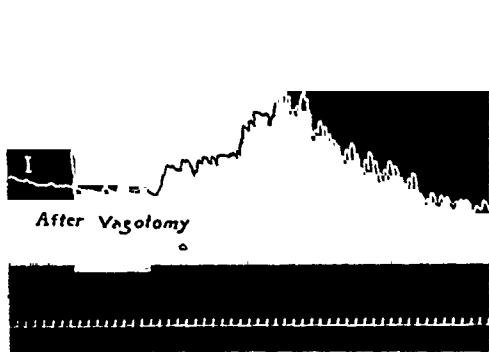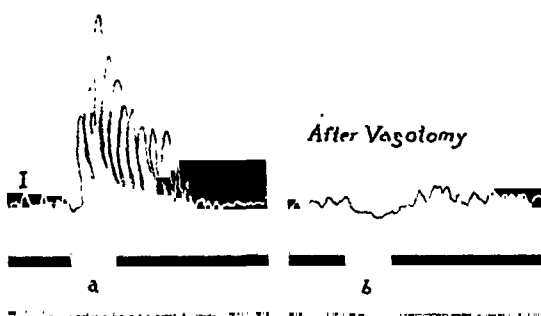
Fig. 6   Fig. 7

Fig. 6. Kymograph tracing showing the delayed excitatory response of the small intestine (I) after both vagi have been sectioned.   Time in 6 seconds.

Fig. 7. Kymograph tracings showing the immediate excitatory response of the small intestine (I) in the tracing (a) which was abolished after vagotomy (b).   Time in 6 seconds.

observed (fig. 7).   The tone of the intestine and colon was raised promptly with increased movement which lasted for the period of stimulus or considerably longer; the latency never exceeded 7 seconds and it returned to the normal quiescent state and tone promptly.   This was easily repeatable and no period of rest was found to be necessary.   These responses were not intermingled with the delayed response in each individual experiment. In the same animal this immediate response was obtained from points situated dorsally or caudally to the reactive region for the delayed response. However, as often only a limited area was stimulated in each experiment, there was usually only one type of response observed.   In rare cases, a combined response of immediate and then delayed excitation with a short interval of quiescence was encountered.   These two types of responses, even when they occurred together, could be differentiated without dif-

ficulty. In 2 cats the mammillary body itself was stimulated and no response was observed. Vagotomy in 3 of these animals (table 1) would invariably abolish the immediate response of the small intestine (fig. 7).

In 4 cats, after a delayed excitatory response was demonstrated, the spinal cord was cut at the upper thoracic segment. Stimulation of the same region did not again bring about similar response. Seven chronic cervical spinal cats were stimulated, 3 of which definitely showed no delayed response. In 2 of these animals an immediate excitatory response was observed on stimulating the hypothalamus at the infundibular level, which disappeared after vagotomy. In the 4 others, only a slight but delayed increase of tone and motility of the intestine or colon was demonstrated. In one of these animals, vagotomy was done and the slight response remained unobscured.

TABLE 1

Different types of gastro-intestinal responses following stimulation of the hypothalamus

LEVEL STIMULATED (NUMBER REFERS TO FIGURES)	TOTAL NUMBER OF ANIMALS	NUMBER OF ANIMALS SHOWING:				
		Delayed excitation following unchanged activity (a), inhibition (b) or excitation (c) during stimulus			Only excitation during stimulus	Negative result
		(a)	(b)	(c)		
1	12	3	3	0	1	5
2	15	9	3	1*	0	2
3	16	5	4	0	2†	5
4	10	2	0	0	4	4
Pituitrin (4 pressor units)	5	2	1	0	0	2

* Section of both vagi abolished the immediate excitatory component of the response.
† Section of both vagi abolished the responses.

DISCUSSION. It is easy to understand the mechanism of this immediate excitatory response. Since its onset is abrupt and prompt and its effect lasts as long as the stimulus or only slightly longer, a neural mechanism is definitely suggested. The fact that it is also found in spinal cats and disappears after vagotomy clearly indicates a vagal effect. By and large, this is very similar to the acute response reported by Beattie (1932a) on the stomach and by Masserman and Haertig (1938) on the small intestine; the former found that the gastric effect is abolished after section of both vagi.

As to the delayed excitatory response, its mechanism appears to us to be different. It has a latent period of 40 to 60 seconds, its onset is gradual and its effect lasts for several minutes after the cessation of the stimulus. The responses on the stomach obtained by Beattie and Sheehan (1934) and

Heslop (1938b) were all delayed. The former stated: "There was a latent period of approximately 30 seconds between the commencement of stimulation and the first rise in intragastric pressure. With direct vagal stimulation the latent period was only a few seconds (never more than 10 seconds)." These authors regard all the excitatory responses as vagal. Since our responses were obtained in several vagotomized cats, we cannot suggest that these effects are identical. However, it is to be pointed out that the central effect differs distinctly in many ways from the peripheral vagal effect (figs. 3 and 1, Beattie and Sheehan, 1934), and the difference is strikingly similar to that shown in figure 5 and figure 7 presented here. Furthermore, Heslop in his work on gastric secretion (1938a) reported that anterior hypothalamic stimulation remained successful in accelerating the flow of gastric juice and raising the acidity in 2 chronic vagotomized cats. However, his suggestion that these vagal impulses are reaching the stomach via an alternative route, needs some additional experimental evidence.

It is known that secretion of the posterior lobe of the pituitary gland may be liberated following stimulation of the hypophyseal stalk (Haterius and Ferguson, 1938) and that of the anterior hypothalamus (Clark and Wang, 1939). Though the effect of various fractions of the posterior lobe secretion on the gut is reported to be conflicting (Geiling, 1926) and inconstant (van Dyke, 1936), our own results seem to indicate that it has an excitatory effect on the small intestine and colon (table 1).

On the other hand, in 7 healthy chronic spinal cats, none showed a definite delayed excitatory response on stimulation of the anterior hypothalamus, though in 4 of them (one vagotomized) its presence is suggestive. It is true that many of our stimulation experiments on the normal cats yielded negative results. The absence of a single convincing positive response, however, would certainly make us hesitant in suggesting that this delayed excitation is related to the hypophysis. At the same time, the absence of a similar response in these spinal, but vagus-intact cats would show that this response is not a simple vagal effect. Until further evidence as to its mechanism is available, we deem it wise not to assume that the delayed excitation is effected through the "parasympathetic center" in the anterior hypothalamus (Beattie, 1932b).

SUMMARY

The hypothalamus of more than 50 fasting cats was stimulated by faradic current under light ether or chloralose anesthesia. Its effect on the gastro-intestinal tract was observed through a transparent window or recorded by the balloon method.

Stimulation of the hypothalamus anterior to the infundibular region yielded immediate blanching and occasional inhibition, followed by a

marked excitatory response. Its onset was slow and gradual, and its effect lasted for several minutes. Section of both vagi did not abolish this effect. Its possible mechanism is discussed.

Vagal effects on the gut were obtained when the hypothalamus at or behind the infundibular level was stimulated. Such responses could be elicited in chronic spinal cats and were abolished after bilateral vagotomy.

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THE DISTRIBUTION OF GLUCOSE BETWEEN CELLS AND SERUM: FURTHER EXPERIMENTS WITH HIGH CONCENTRATIONS OF GLUCOSE[1]

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In experiments previously reported (5), in which solutions of glucose were added to blood, it was found that when the concentration of glucose in the diluted blood was less than 1500 mgm. per cent, the glucose became evenly distributed between the water of cells and serum, and the added water was partitioned between the two phases in the same proportion as water added without glucose, or as solutions of urea. The increase in the volume of the corpuscular water was immediate, and almost proportionally equal to that of the water of the serum. When solutions containing higher concentrations of glucose were employed the cells swelled more slowly and when equilibrium had apparently been attained the increment of water in the cells was proportionally much less than the increment in the serum. Furthermore, with such solutions hemolysis did not occur. Since it was found that osmotically significant amounts of base did not escape from the cells, it was inferred that the permeability of the cell membrane was altered in some manner so as to limit the entrance of glucose into the cells.

When a drop of blood was added to 3 cc. of glucose solutions of varying concentrations, hemolysis occurred in those in which the concentration was less than 2300 mgm. per cent. This variation in the concentration of glucose which prevented the free penetration of glucose into the cell indicated that the alteration in permeability is not entirely due to the increase in the glucose concentration. It is, nevertheless, intimately associated with it, since there was no variation when solutions containing low concentrations of glucose were osmotically fortified by the addition of non-electrolytes (sucrose and lactose) and electrolytes to which the cell membranes are impervious. Eisenman, Hald and Peters (1) have shown that in the circulating blood, under conditions possibly associated with metabolic activities of the cells, the base in the cells may change without affecting the distribution of water between cells and serum. The latter is apparently

controlled by the osmotic pressure of the serum, in which electrolytes are the chief osmotically active components. Similar paradoxical phenomena were not encountered when blood was properly handled *in vitro*. Halpern (4) has demonstrated also that if blood is kept at or below room temperature cellular metabolism of glucose and associated transfers of phosphate proceed too slowly to be significant in the intervals of time involved in the experiments with glucose under discussion.

To define more exactly and possibly to explain the anomalous behavior of blood cells to glucose the experiments here reported were carried out. It was also hoped that they might throw some light on the change in the response of the tissues to glucose when the concentration of this substance in the blood becomes extremely high. The experiments of Wierzuchowski (8) would suggest that when the blood sugar exceeds a certain maximum figure glucose ceases to enter the tissues.

PROCEDURE. To a portion of human venous blood which had been defibrinated by stirring with a glass rod, glucose solution, in concentrations and amounts indicated in columns 2 and 3 of table 1, was added. After the diluted blood, thoroughly mixed, had stood at room temperature for from 15 to 30 minutes, the cell volume of the blood and the concentrations of glucose in whole blood and serum were determined. In most instances these determinations were repeated after an interval of 3 to 5 hours; in 3 instances the final determinations were made after intervals of 26, 48 and 50 hours respectively. In these cases, the blood was kept most of the time in a refrigerator at 5°C. The cell volume of the original was also measured. With the assumption that 72 per cent of the volume of the cells and 93.5 per cent of the serum were composed of water, the concentrations of glucose in the water of the cells and the serum were estimated. In another series of similar experiments, blood and serum were analyzed for inorganic phosphate instead of for glucose. In 2 experiments, in which dry glucose, instead of glucose solution, was added to blood, the measurements of cell volume and glucose concentration were repeated at 5 and 8 hour intervals. In some experiments water, instead of glucose, was added, and the cell volume, serum proteins and base, cell volume and dry weight, or just cell volume determined.

Analytical methods. Glucose determinations were made on a Somogyi (7) filtrate after previously diluting the whole blood or serum to a convenient degree. The Shaffer-Hartman-Somogyi (6) titration method was used.

The Fiske and Subbarow (2) method was used for the determination of inorganic phosphorus. A micro modification devised by Kydd and employed in this laboratory for many years was found quite satisfactory. One-half cubic centimeter of blood or serum was precipitated with 3 cc. of 10 per cent trichloroacetic acid, and to 1 cc. of the filtrate, 0.7 cc. of

water, 0.2 cc. 2.5 per cent ammonium molybdate in acid, and 0.1 cc. sulfonic acid reagent were added.   Duplicates were analyzed in all cases.

Serum proteins were determined by the macro-Kjeldahl method.

Methods described by Hald (4) were used in the estimation of sodium and total base of serum.

Cell volumes were measured in a Daland hematocrit tube centrifuged at 1500 r.p.m. for one hour.

Calculations. The increases of cell and serum water were calculated as previously described (5).   Estimation of total osmolar equivalents is discussed below.

RESULTS AND DISCUSSION.   The results of the experiments in which glucose was estimated are presented in table 1.

As in previous experiments (5), when glucose solutions were added to blood so that the concentration of glucose was less than 1500 mgm. per cent, the added water and the glucose distributed themselves immediately and almost equally between the cells and serum.   With higher concentrations of glucose water entered the cells quite slowly and hemolysis was never induced.   Analyses revealed that with the less concentrated solutions the concentrations of glucose per unit of water in cells and serum were approximately equal within 30 minutes.   With the more concentrated solutions there was decided inequality in the distribution of glucose, with a tendency to approach equality as the water in the cells gradually increased. When dry glucose was added to blood this approach to equality was accelerated (expts. 17, 17A, 18, 18A and 18B).   Although inequality of distribution was noted with a whole blood sugar concentration of 1900 mgm. per cent, in other experiments the glucose of cell water reached 5970 and 6700 mgm. per cent.   There is not, then, a limit to the amount of glucose which can be made to enter the cells, but rather a change in the rate at which it enters.

In part the differences in the reactions to weak and strong glucose solutions seem to depend upon the relative speeds with which glucose and water diffuse across the membrane.   This is apparent from the fact that as the concentration of glucose is increased, initial contraction of the cells is noted. The failure to attain final equality of distribution might on this account be ascribed merely to the fact that the periods of observation were not sufficiently prolonged.   There are, however, arguments against this explanation.   First and foremost is the fact that cells cannot be hemolyzed by these strong solutions and, therefore, presumably will not admit unlimited amounts of glucose and water.   In the few experiments in which analyses were made as much as 26 and 50 hours after the addition of the glucose solution, the rate of entrance of glucose into the cells is less at the later times than shortly after adding the glucose, indicating, perhaps, that the rate is approaching zero.   In addition, when the experiments with dry

TABLE 1

Results obtained when glucose in solution and dry was added to whole blood

The calculations are described in the text

EXPERI-MENT NUMBER	SOLUTION ADDED TO BLOOD		INCREASE OF WATER IN		GLUCOSE OF WATER OF		RELATIVE OSMOLAR CONCENTRATION*		TIME AFTER ADDING GLUCOSE
	Volume per cent	Glucose	Cells	Serum	Cells	Serum	Cells	Serum	
	cc.	per cent	per cent	per cent	mgm. per cent	mgm. per cent	per cent	per cent	hours
1	30	3	33	37	920	1030	91	92	0.25
2	40	3	42	52	1030	1212	89	88	0.25
3	20	11	4	31	1295	2480	122	129	0.25
4	40	11	−8	80	1530	4470	138	137	0.25
5	50	11	2	91	2300	4960	142	147	0.25
6	50	11	−1	108	2160	5120	142	146	0.50
7	50	11	−13	106	1650	4880	146	142	0.25
8	60	11	−8	115	1560	5400	141	149	0.25
9	62.5	11	−8	134	2130	5600	136	148	0.25
10	66.7	11	−8	131	1900	5700	145	152	0.25
11	66.7	15	−18	140	2980	7600	182	186	0.50
12	70	15	−29	158	1600	7900	177	189	0.25
13	75	15	−17	139	3300	7820	186	192	0.50
14	83.2	15	−23	164	3100	8600	192	202	0.25
15	70	15	25	115	5600	7350	185	183	2
6A	50	11	26	86	3170	4880	140	146	3
7A	50	11	29	77	3450	4400	139	141	3.25
9A	62.5	11	27	108	3650	5200	149	146	4.75
10A	66.7	11	27	112	3400	5400	143	150	5.50
11A	66.7	15	12	122	4930	7400	185	185	5
16	70	15	3	130	4650	7600	179	187	6
13A	75	15	8	127	4900	7690	190	191	5.25
14A	83.2	15	−13	162	4400	8600	204	200	4.25
12A	70	15	0	135	4200	7680	178	187	9
12B	70	15	11	132	4750	7400	184	182	26
16A	70	15	17	125	4800	7550	174	187	31
16B	70	15	24	121	5440	7380	170	185	48
15A	70	15	37	108	5970	7240	184	185	50
17	Dry glucose added		−21	16	3980	6000	200	196	0.33
18	Dry glucose added		−25	30	5000	8250	220	227	0.50
17A	Dry glucose added		−2	7	5050	5500	195	193	4.50
18A	Dry glucose added		−10	18	6300	7600	227	223	5
18B	Dry glucose added		0	10	6700	7300	222	226	9.25

* Osmolar concentrations are expressed in per cent of the original osmolar concentrations, with the assumption that the latter were equivalent to a 5.50 per cent solution of glucose (for details see text).

glucose are compared with those in which glucose solutions were added, it will be seen that in the former glucose and water are almost equally distributed, while in the latter there is a large discrepancy, although the con-

centrations of glucose in the dry glucose experiments are as high or higher than they are in the experiments with glucose solutions.[2]

At any concentration of glucose in the added solution the amounts of glucose and water that enter the cells seem to be, to some extent, independent of the degree of dilution. This is especially evident in the late observations on the 11.0 per cent groups (expts. 7A and 10A). The cells increase 29 and 27 per cent with cellular glucose concentrations of 3450 and 3400 mgm. per cent in experiments in which the blood was diluted 50 and 66.7 per cent. In dilute solutions the increase of cell volume is directly related to the degree of dilution. Standing alone, this could be taken as direct evidence that the permeability of the cell membrane to glucose is the limiting factor. But the dry glucose experiments, if considered alone, would appear to indicate that the permeability to glucose is not an important factor, and that, possibly, the permeability to water is.

In this connection the previously reported (5) slight differences in the increments of cellular and serum water following the addition of water,

[2] Other experiments were designed to test the possibility that the inequality of glucose distribution was merely a function of slow diffusibility of glucose in high concentration. Blood hemolyzed with saponin was placed in one chamber of a Lavietes filter, serum in the other, and glucose, in solution or dry, added to either side. No pressure was exerted on the intervening cellophane membrane. It was found that glucose in high concentrations diffused very slowly, and more slowly in hemolyzed blood than in serum or in saline. For example, in one set of experiments glucose solution was added to the hemolyzed blood in one filter, to the serum in a second, and in the third serum plus glucose solution was separated by a membrane from serum. The amounts added were about the same. At the end of 8 hours at room temperature, the hemolyzed blood in the first filter contained, per unit of water, more than twice as much glucose as its opposing serum, 6300 mgm. per cent compared with 3000 mgm. per cent; the blood in the second contained less than half as much as its opposing serum, 3250 mgm. per cent compared with 7300 mgm. per cent; but the glucose had diffused with considerable more speed in the filter containing only serum, for the serum which had contained none, now contained 4200 mgm. per cent glucose per unit of water, compared with 5700 mgm. per cent on the other side of the cellophane membrane. At these concentrations equality of distribution in serum and whole blood was not attained in 72 hours, but was in one experiment lasting 96 hours. If both sides of the filter contained serum, glucose distribution was still unequal at the end of 72 hours, but the disparity was not nearly so great as when one chamber of the filter contained hemolyzed blood. When saline was used instead of hemolyzed blood, distribution was almost equal in 24 hours. At lower concentrations, 1500 mgm. per cent, there was equal distribution in water of hemolyzed blood and serum in 8 hours. No significant difference was observed between glucose added in solution and glucose added dry. Although the absence of this difference might invalidate these experiments as being directly analogous, the slow rate of diffusion cannot be disregarded. On the other hand, the comparatively large surface area of the cells in whole blood should certainly facilitate diffusion, compared with the rather small area of cellophane in the filters. Until a more comparable set of conditions can be devised, the factor of the rate of diffusion of glucose cannot be evaluated.

urea solution and glycerol solution to blood become pertinent. The addition of water to whole blood was repeated in a number of experiments and the percentage dilutions of cells and serum were calculated from the observed cell volumes, the water contents of whole blood and serum, the serum protein concentrations, or from serum base concentrations. The results are presented in figure 1, and completely confirm the previous

Increase of water of serum, per-cent
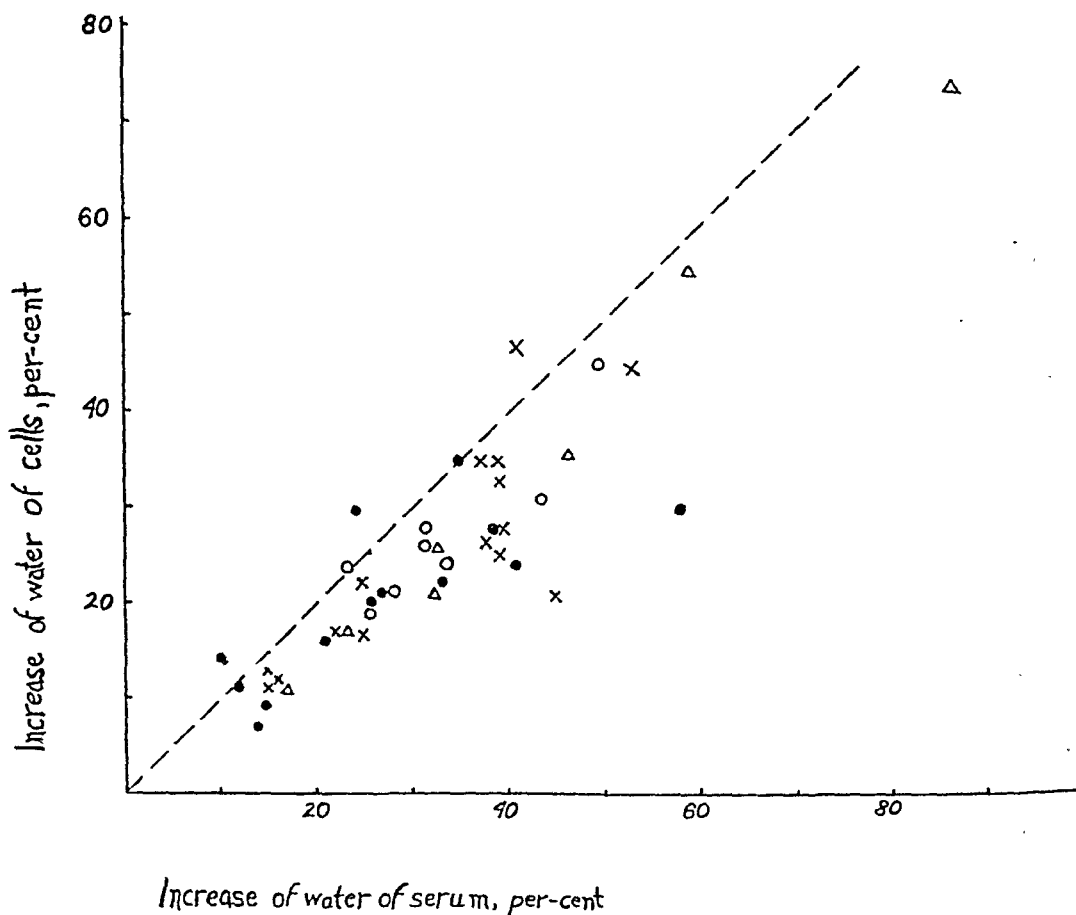
Fig. 1. Percentage increases in water of cells and serum when water was added to blood. Calculations were made from cell volume, ●, from cell volumes and determined water contents, ×, and from cell volumes, serum proteins and serum bases, ○. Certain of the experiments, △, have been presented in a previous publication (5). The broken line is placed at an angle of 45°.

observations. The failure of the cellular water to increase as much, proportionally, as the serum water is quite definite, and is associated with an apparent osmotic anomaly not found when solutions of electrolytes are added to blood *in vitro* (1), for the ratio (water of cells$_2$/water of cells$_1$: (base of serum H$_2$O$_1$/base of serum H$_2$O$_2$)) is not unity, but closer to 0.95.

Because, however, of experiments already cited, and because of the ease with which hemolysis is induced with dilute glucose solutions, and es-

pecially since cells at first shrink in strong glucose solutions, it is hardly permissible to assign the apparently changed permeability merely to the addition of water. For the present it must be surmised that both glucose and water control the penetration of glucose into the cell, and that to neither alone can be assigned a position of prime importance in an interpretation of the results.

With the concentrations of glucose in water of cells and serum known, and since electrolytes do not cross the cell membrane, the osmolar concentrations of the two phases can be compared at any point in the experiments. Serum and cells are isotonic with a 5.5 per cent solution of glucose, and therefore presumably contain the same osmolar equivalents as such a solution. If it be assumed, then, that cells and serum originally contained an osmolar concentration of 100 per cent or 5500 mgm. per cent of glucose, and due correction be made for changes in volume of the two media, the total osmolar concentration of the final contents of cells and serum can be estimated by merely adding the analytically determined concentrations of glucose in comparable terms of osmolar equivalents. The experiments with water added would indicate the possibility that the electrolytes in the cell change their osmotic activities, but this possibility should offer no insurmountable objection, since such a change would almost certainly be slight, and so affect little the results of the calculations, which are only approximations. It is evident from these estimations (table 1, columns 8 and 9) that at every stage of the transfers of water and glucose osmotic equilibrium is maintained, even when the distribution of glucose is most disparate.

Analyses for inorganic phosphorus revealed no changes that could not be attributed merely to changes in the relative volumes of cells and serum. The passage of glucose across the cell membrane, therefore, involved no demonstrable reactions with phosphorus such as are noted when metabolism of carbohydrate in the cells is active.

Such unequal distribution of glucose has been advanced as evidence that there is "bound water" in cells, that is, water that is not available as solvent. Dilution of blood with water seems to strengthen this concept. Nevertheless, although no alternative explanation for the anomalous behaviors of glucose and water has been found, the weight of accumulated evidence is against such a concept, and the phenomena described are quite incompatible with it. The estimations of osmotic pressure prove that within the limits of error of the methods employed, all the water in cells and serum is available for the solution of solutes in general. In dilute glucose solutions there is complete equality of glucose distribution. In strong solutions the degree of inequality varies with the concentration of glucose solution added and the lapse of time. That factors other than these are involved, however, is evident from the fact that while the addi-

tion to blood of 15 per cent glucose solution results in a greater inequality than does the addition of 11 per cent glucose, the inequality of distribution consequent on the addition of dry glucose is much less than that observed with either of these solutions.

SUMMARY

When concentrated solutions of glucose are added to blood there is a marked discrepancy between the amount of glucose taken up by the cells and that remaining in the serum. The disproportion tends to diminish with time, but does not disappear completely. The limiting factors in the free penetrability of the cell membrane to glucose are discussed but are not precisely defined. It is demonstrated, however, that despite the discrepancy in the distribution of glucose, osmolar equality between cells and serum is still maintained.

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THE RELATIONSHIP BETWEEN DIFFERENTIAL PRESSURE AND BLOOD FLOW IN A CORONARY ARTERY[1]

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In 1934 Wiggers (1) proposed a procedure which with certain modifications was used to study the phasic variations of flow in all three coronary arteries (2, 3, 4). This procedure involved the subtraction of the peripheral coronary arterial pressure from the central coronary pressure. It yielded a curve (differential pressure curve) whose ordinate values were assumed to be at each instant proportional to the rate of coronary flow.

The present communication is concerned with an experimental investigation of this assumption. For this purpose a procedure was devised, including the development of a new type of flow meter, by which it is possible to register both the peripheral coronary pressure and the phasic changes of inflow into the coronary artery at predetermined heads of pressure. By comparing the rate of inflow with the corresponding differential pressure (difference between perfusion pressure and peripheral coronary pressure) the relationship between differential pressure and flow has been determined.

METHODS. *Apparatus.* The flow meter (see fig. 1) consists of a reservoir, B, containing about 5 cc. of blood, which connects through a three-way stopcock, S, and lead tube with the artery to be perfused. The reservoir is expanded at its upper end into a chamber, A, filled with air at the pressure at which it is desired to perfuse the artery. A sensitive optical manometer, MF, incorporated as an integral part of the chamber records the slight decline of the air pressure as the blood leaves the reservoir, the rate of decline of pressure indicating the perfusion rate. The meter is rigidly supported by a heavy bar fastened to M.

The peripheral coronary pressure and perfusion pressure are recorded by a Gregg pressure manometer, MP, connected with the lead tube. Both manometers, MF and MP, are fitted with special rubber membranes,—

that of the flow meter being 0.006 inch thick and that of the pressure manometer 0.020 inch thick, and supplied with +1.0 dioptre planoconvex mirrors.   At a projection distance of 4 meters the former has a sensitivity of 20 mm. for 1 mm. Hg with a natural frequency of 150 per second and the latter a sensitivity of 40 to 60 mm. for 100 mm. Hg with a natural frequency of 200 to 250 per second.

Fig. 1. Diagram of flow meter.   Description in text

EXPERIMENTAL PROCEDURE.   Successful experiments were performed on eight dogs with essentially the same results.   After anesthetization with morphine and sodium barbital and institution of artificial respiration the heart was exposed and a short length of the ramus descendens together with a suitable side branch dissected free.   The animal's blood was rendered noncoagulable with heparin (75 units per kgm.) and chorazol fast pink (80 mgm. per kgm.).   The side branch of the coronary was cannulated

and connected with the flow meter as shown in figure 1 and another pressure manometer similar to the one described above was connected with the aorta.

Operation of apparatus. The reservoir is partially filled with blood (see fig. 1) and the pressure in the chamber is raised to the desired level (20 to 200 mm. Hg). After recording a few heart beats the main coronary vessel is clamped (at *C*, fig. 1), and the stopcock in the flow meter turned to the position shown in figure 1. As the blood enters the artery the chamber pressure drops slightly resulting in a progressive movement of the flow meter and coronary pressure beams (from left to right) across the photokymograph lens. By varying the size of the chamber its rate of pressure drop can be generally kept below 1 mm. Hg per heart beat and the sensitivity so controlled that the flow beam moves 12 cm. (the width of the sensitive paper) in 3–5 heart beats.

To obtain the data necessary for constructing the differential pressure curve for comparison with the flow determinations the stopcock (*S*, fig. 1) is rotated 90° to the right, the coronary artery is clamped for 8–10 beats and the peripheral coronary pressure recorded with the pressure manometer (*MP*, fig. 1). The correct systolic value of the peripheral coronary pressure is recorded by intermittently clamping the coronary artery during systole at a rate slightly asynchronous with the heart rate. (See reference (3) for details.)

The flow meter is calibrated during an experiment by recording the amplitude of the deflection at a series of different perfusion pressures when 0.5 cc. of fluid is driven into or out of the reservoir with a tuberculin syringe.

Adequacy of the meter. The meter was tested as follows: 1, the meter was filled and connected by lead tubing to a 10 cc. syringe, the plunger of which (activated by a mechanical device) moved in and out of its barrel with a sine wave motion and caused fluid to enter and leave the meter. The plunger movements were recorded simultaneously with the flow meter beam. In figure 2A with the plunger, *P*, making 12.5 double strokes per second (stroke volume 1 cc., *M*) the lag of the meter is less than 0.005 second while at lesser frequencies the lag is too small to read. 2. The flow from the meter (at various air chamber pressures) was started and stopped abruptly either by rotation of stopcock, *S*, or by an intermittent compression of a rubber tube by an electromagnetic clamp (fig. 2B). There is no evidence of appreciable lag or overshooting.

RESULTS. In segment A of figure 3 the aortic pressure, *AP*, and coronary pressure, *CP*, are recorded simultaneously.[2] At time *K* the central coronary artery was clamped and the coronary manometer began to

[2] To facilitate inspection vertical lines A, B, C, D, A¹ have been drawn through simultaneous points on the curves.

record the pressure in the coronary artery distal to the clamp. At point S the stopcock S (fig. 1) was turned so that blood under pressure of 113 mm. Hg (slightly less than aortic systolic) entered the artery from the reservoir. The coronary artery manometer beam swings up and begins recording

A

B
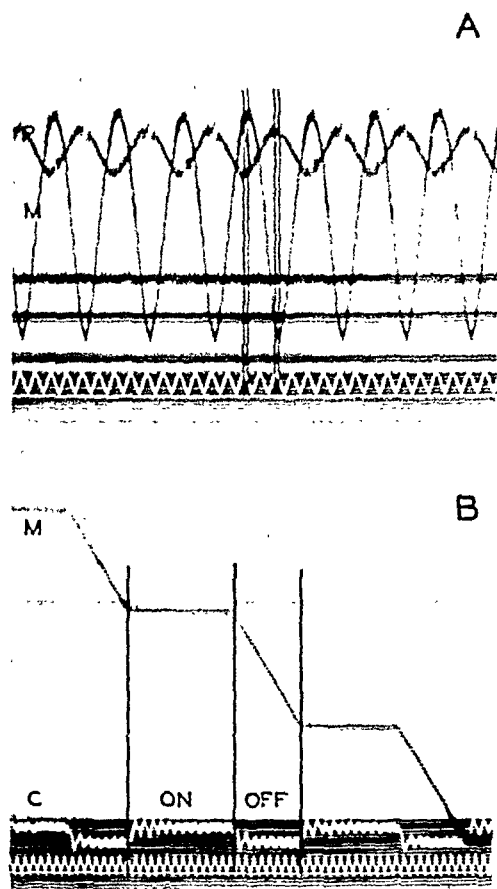
Fig. 2. A. Test of adequacy of flow meter. M, record written by meter, upward movement = inflow of blood into meter; P, record written by a mirror attached to moving plunger of a syringe connected by a lead tube with the meter, downward movement = expulsion of blood from syringe into meter; time, $\frac{1}{5}$ second; lag, indicated by vertical lines = 0.005 second.

B. Test of adequacy of flow meter. Meter outflow interrupted by an electromagnetic clamp arranged to compress a short length of thin walled rubber tube through which fluid leaves meter. M, record written by meter; C, record of signal in series with the clamp, upward movement indicates compression of tube, downward movement release. Time, $\frac{1}{50}$ second.

the perfusion pressure, PP. Within a short interval the flow beam, previously off the field, begins to cross the record in an irregular slanting line, F. A definite flow exists throughout the cardiac cycle.

During systole the rate of flow begins to diminish at A^1 (i.e., at the

beginning of the isometric contraction period), decelerates further to B and then remains relatively constant from B–C.

During diastole the rate of flow starts to increase at C (coincident with the decline of the peripheral coronary pressure curve) and reaches a maximum at D, i.e., at about the diastolic valley of the peripheral coronary
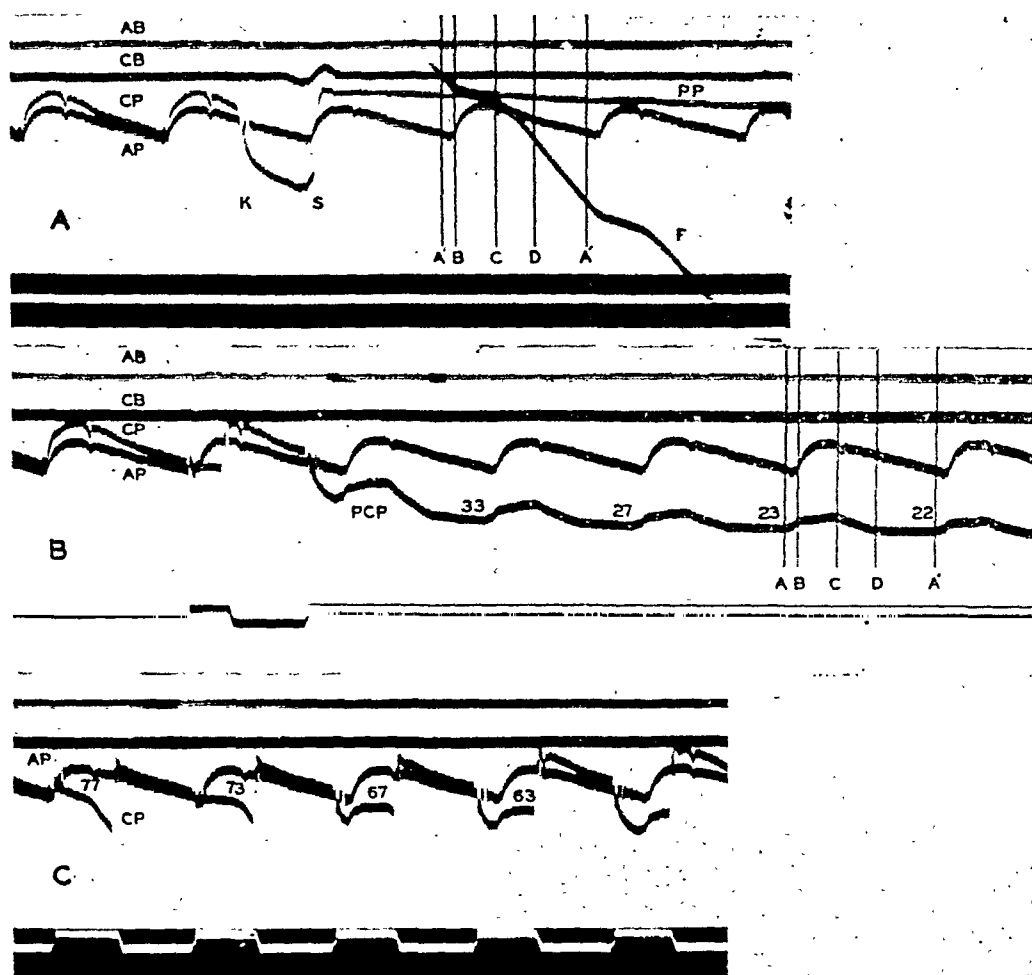
Fig. 3. Comparison of differential pressure with flow. A. Record of flow. B. Determination of diastolic peripheral coronary pressure. C. Determination of systolic peripheral coronary pressure. AP, aortic pressure; CP, coronary pressure; AB and CB, base lines for aortic and coronary manometers respectively; PP, perfusion pressure, measured with coronary manometer; K, time of clamping main coronary vessel; S, instant when stopcock (S) turned to position shown in figure 1; figures indicate pressures in millimeters of mercury. For other details see text.

pressure curve. In late diastole (D–A^1) the rate of flow is essentially constant.

Comparison of the recorded flow curve with the flow curve constructed from the differential pressure curve. For this purpose the necessary tracings and reconstructions of figure 4 have been taken from figure 3.

Curve F (solid line), figure 4B, is a tracing of the recorded flow curve in figure 3A.   Curve F (dashed line)[3] is the theoretical flow curve reconstructed from the differential pressure curve, DP.   The latter differential curve was constructed as follows: The peripheral coronary pressure curve of figure 3B was raised to its proper ordinate value of 67 mm. Hg (cf.
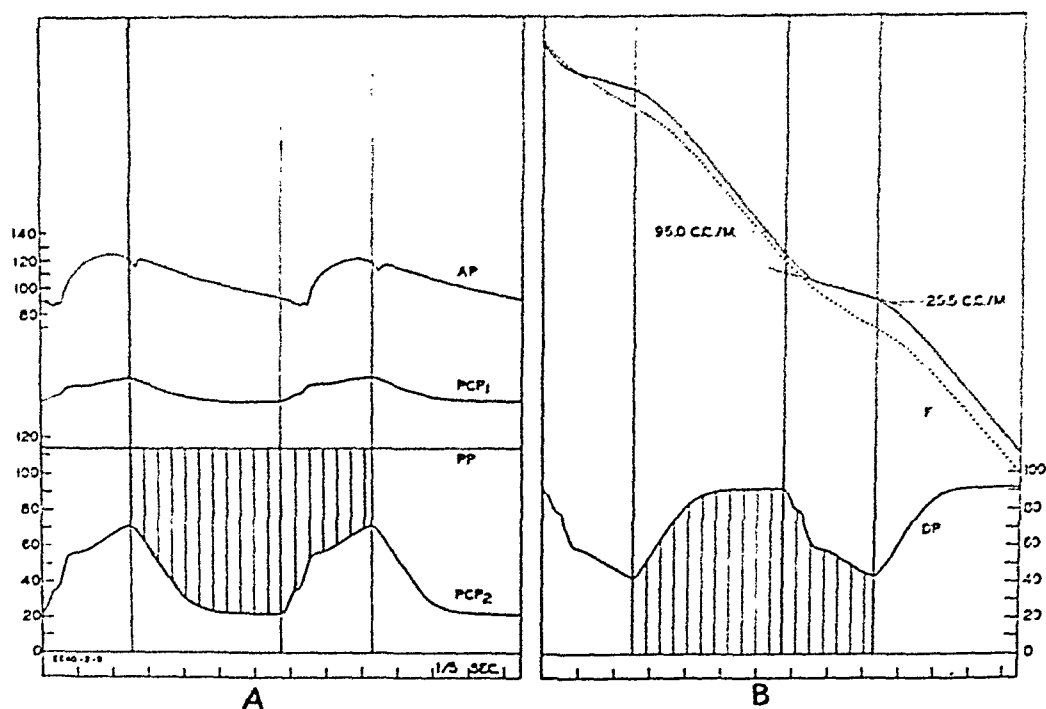
Fig. 4. A. Calculation of differential pressure. AP, aortic pressure, calibration at left; PCP_1, tracing of peripheral coronary pressure curve: PCP_2, peripheral coronary pressure curve (PCP_1) enlarged to correspond to aortic pressure scale, calibration at left; PP, line corresponding to level of pressure at which coronary artery was perfused; vertical lines, differential pressure (perfusion pressure minus peripheral coronary pressure).

B. Construction of theoretical flow curve from differential pressure curve and comparison with recorded flow curve. F, flow curves, solid line = recorded curve, dashed line = constructed curve; DP, differential pressure, from A, scale at right. Theoretical flow curve constructed so that slope at each point is proportional to the corresponding differential pressure, the slope at the end of the first diastole being made equal to that of the recorded curve; figures correspond to rates of flow in latter part of diastole and midsystole respectively, in recorded curve.

fig. 3C) and appears in figure 4A as PCP_2.   This was then subtracted from the constant infusion pressure of 117 mm. Hg (PP of fig. 4A).   The difference (vertical shading) (fig. 4A, B) represents the moment to moment

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[3] Arbitrarily, the reconstructed and recorded flow curves were drawn with the same slope at the end of diastole while the slope at all other points was made proportional to this slope.

differential pressure between the perfusion pressure and the peripheral coronary pressure.

While it is realized that considerable error is involved in such reconstructions still if the differential pressure curve gives a reasonably correct picture of the moment to moment flow then a reconstructed flow curve drawn from it so that the slope at each point is proportional to the differential pressure should approximate the contour of the recorded flow curve. Certain differences are at once obvious in the reconstructed flow curves: 1, the flow during systolic ejection is greater and is more gradually reduced; 2, minimum flow is reached later, i.e., at the peak of the peripheral coronary pressure; 3, the acceleration of flow is less rapid during early diastole. On the other hand, at the instant corresponding to the peak of the peripheral coronary pressure curve the slope of the recorded curve has increased sufficiently to approximate that of the reconstructed curve.

In making these comparisons one assumption made in previous work required experimental validation, i.e., the peripheral coronary pressure curve was considered as that pressure which at each moment would just not cause fluid to flow through intramural vessels. To test the accuracy of this concept for two points on the flow curve, i.e., the maximal and minimal points, the coronary bed was perfused with blood at pressures approximately equal to these two values. The resultant flow curves and PCP curves redrawn from the original records are presented in figure 5. At perfusion pressures approximately equal to the diastolic minimal value of the peripheral coronary pressure (12 mm. Hg, cf. lines PP_2, fig. 5) there is a to and fro movement of blood but no forward flow (line F_2). When the perfusion pressure is raised to approximately the systolic maximal value (82 mm. Hg, line PP_1) the diastolic flow is considerable (line F_1) but the systolic flow is practically zero except for the isometric contraction period.

From these findings the conclusion is drawn that the maximal and minimal values of the peripheral coronary pressure curve equal those heads of pressure which will just not cause fluid to flow into the coronary bed during the middle of systole and the latter part of diastole.

The normal relationship between differential pressure and flow. In previous publications the rate of intramural flow at each instant was considered proportional to the differential pressure (head of pressure minus peripheral coronary pressure). Measurements[4] made on 125 records in different experiments showed that the systolic rate of flow per millimeter of mercury differential pressure is consistently less than the diastolic for

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[4] The points chosen for measuring the systolic and diastolic flows were the latter portions of B-C and D-A^1 (fig. 3) during which the peripheral coronary pressure curve is changing minimally. In some records the systolic flow was determined at the peak of the PCP curve.

the same heart cycle (cf. table 1). For example, in figure 4 during systole with a minimal differential pressure of 43 mm. Hg the flow is 25.5 cc. per minute or 0.59 cc. per minute per mm. Hg, while during diastole the flow is 1.04 cc. per minute per mm. Hg.
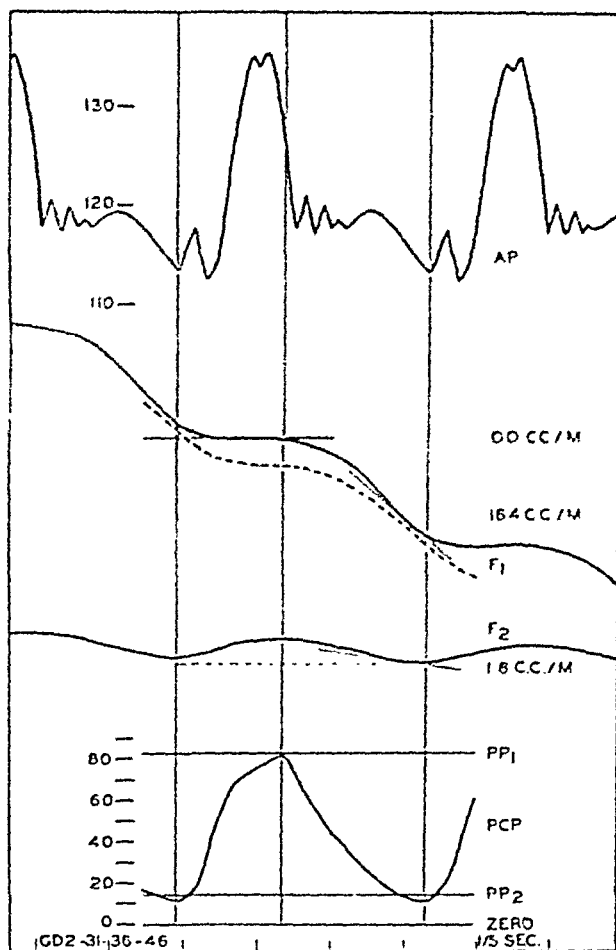
Fig. 5. Tracings of curves to show rates of flow when coronary artery perfused at pressures corresponding to systolic and diastolic levels of peripheral coronary pressure. AP, aortic pressure, calibration at left; F_1, flow curve when coronary artery perfused at a pressure equal to the systolic value (PP_1) of the peripheral coronary pressure curve (PCP); F_2, flow curve when coronary artery perfused at a pressure equal to the diastolic value (PP_2) of the peripheral coronary pressure; calibration, lower left, refers to peripheral coronary pressure curve and lines indicating perfusion pressure; figures at right of flow lines indicate the rates of flow at mid-systole and late diastole for the two flow curves.

To study further the relationship between flow and differential pressure, the coronary bed was perfused at a variety of pressures and the rates of flow during the middle of systole and during the latter part of diastole compared with the resulting differential pressures. If the rate of flow

per millimeter of mercury differential pressure were always constant a plot of the values should fall along a straight line passing through the origin. Figure 6 is a graph of the results of a typical experiment. Despite the considerable spread of points, it is obvious that 1, the systolic flow is generally less than the diastolic for a given differential pressure; 2, above 20–30 mm. Hg differential pressure the flow bears a linear relationship to differential pressure during both systole and diastole.

DISCUSSION. The evidence presented in this paper confirms previous observations that the systolic peripheral coronary pressure is generally less than the aortic pressure and that there is a sizable inflow throughout systole when the coronary arteries are perfused with blood at aortic systolic pressure.

TABLE 1

EX-PERI-MENT	AORTIC PRESSURE			SYSTOLE			DIASTOLIC			FLOW, CC. PER MIN. PER MM. Hg, DIFF. PRESSURE	
	Syst.	Diast.	Perf. Press.	P.C.P.	Diff. Press.	Flow	P.C.P.	Diff. Press.	Flow	Syst.	Diast.
						cc. per min.			*cc. per min.*		
1	100	73	90	80	10	9.1	22	68	142	0.91	2.09
	110	85	110	85	25	11.9	22	88	117	0.48	1.35
2	137	95	124	89	35	13.0	35	86	102	0.37	1.19
3	120	85	115	70	45	25.5	20	95	96	0.57	1.01
	150	117	158	78	80	85.0	23	135	186.0	1.04	1.37
4	108	98	110	73	37	2.4	20	90	12.9	0.065	0.143
	122	100	160	80	80	4.4	20	140	18.0	0.055	0.13

The rapid rate of forward flow recorded by the meter during isometric relaxation, exceeding that predicted from the differential pressure curves (fig. 4) and in some instances even exceeding that during the latter part of diastole, probably represents inflow of fluid to fill the larger vessels compressed by the intramural tension during the preceding systole rather than intramural flow. Likewise the retardation of inflow during isometric contraction and midsystole is probably more than the actual slowing of the intramural flow caused by the restriction in the size of the available vascular bed.

The findings presented (see fig. 6) indicate that above a differential pressure of thirty millimeters of mercury there is a fairly linear relationship between differential pressure and coronary inflow for both systole and diastole, but that the inflow during the middle of systole is proportionally less than that during the latter part of diastole. Three possible explanations operating separately or together are advanced to explain the smaller systolic flow: 1, for the same differential pressure the intramural vessels

during systole undergo less expansion with the increased pressure because of their greater extravascular support; 2, the deeper lying vessels are closed during systole thereby reducing the available bed; 3, the potentially
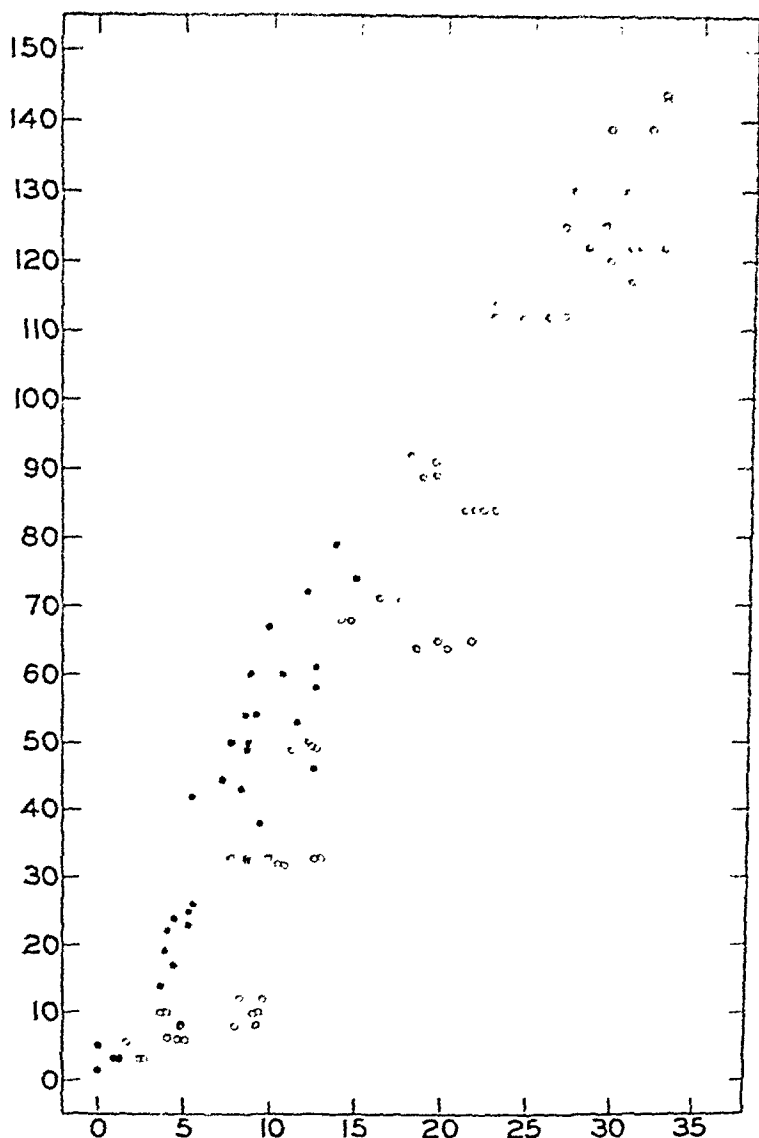
Fig. 6. Plot of the relationship between flow in cubic centimeters per minute (abscissa) and differential pressure in millimeters of mercury (ordinate) for mid-systole and late diastole measured in a large series of records from one experiment. The different differential pressures were obtained by perfusing the artery at various pressures. Circles represent flows in diastole, dots flows in mid-systole.

greater compression force in the deeper layers of the myocardium displaces blood into more superficial vessels.

Since in any one experiment the blood flow and differential pressures vary in the same direction but not always by the same percentage amount,

the method of differential pressures may be used to determine the general contour of the intramural flow but may not be used in a strictly quantitative manner to determine the magnitude of the phasic variations in coronary flow.

SUMMARY

The moment to moment rate of flow into coronary arteries was measured while the vessels were perfused under relatively constant heads of pressure.

Comparison of the rate of blood flow into a coronary artery with the differential pressure (difference between the perfusion pressure and peripheral coronary pressure) shows that: 1, the peripheral coronary pressures indicate the exact time relations of the changes of resistance to flow in the coronary arteries; 2, the systolic and diastolic values of these curves correctly represent the heads of pressure that will just not cause inflow of blood during the respective periods of the cardiac cycle; 3, the differential pressure curves represent the direction and roughly the magnitude of the phasic changes of coronary flow but underestimate the exact value of the moment to moment coronary flow.

The curves obtained indicate that under normal conditions (perfusion of the coronary artery with blood from the aorta) the intramural blood flow will show a sudden retardation with the onset of isometric contraction, during systole forward flow will persist in most hearts, with the onset of isometric relaxation forward flow will rapidly increase and will remain rapid during the latter part of diastole diminishing slightly with the decline of the head of pressure in the aorta.

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EFFECTS OF VISCOSITY, ISCHEMIA, CARDIAC OUTPUT AND AORTIC PRESSURE ON CORONARY BLOOD FLOW MEASURED UNDER A CONSTANT PERFUSION PRESSURE[1]

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The normal coronary inflow together with the alterations in coronary flow induced by various circulatory changes have been studied by the method of differential pressures (1, 2, 3). With the development of a constant pressure flow meter (4), however, it was found that *for normal flow* the differential curve represents the directional changes in flow but does not indicate the exact value of the moment to moment coronary flow. Therefore we have reëxamined various determinants of flow and have compared the recorded alterations of differential pressures with the actual changes of coronary flow.

The operative exposure and experimental methods for recording differential pressures and flow were identical with those previously described (1, 2, 4). The inflow into the coronary artery was measured by the constant pressure flow meter and compared with the existing differential pressures during appropriate control periods and 1, during perfusion of the coronary bed with Locke's solution (decreased viscosity plus slight anoxia); 2, immediately after a temporary period of ischemia of the coronary bed; 3, after augmentation of the cardiac output by increasing the venous return (transfusion of blood or intravenous infusion of Locke's solution), and 4, at different aortic pressures induced by compression of the lower thoracic aorta.

For comparison of the flows, measurements were made of the systolic and diastolic rates of flow and also of the flow per minute per millimeter of mercury differential pressure. For measuring the flows an interval was chosen in systole and another in diastole at which the peripheral coronary resistance was considered to be relatively constant, i.e., for the former at about the latter third of systole and for the latter late in diastole.

RESULTS. These are set forth in figures 1 and 2 and in table 1. The data under any one letter in the table are taken from the curves of the figures of the same letter.

The effect on coronary flow of substituting Locke's solution for the animal's own blood is illustrated in figure 1A and B.   In A, perfusion of the ramus descendens with blood at 115 mm. Hg pressure (slightly under aortic systolic) gives systolic and diastolic rates of flow of 3.15 and 19.4 cc. per minute respectively.   After filling the constant pressure meter, cannula and superficial part of the coronary artery with Locke's solution the rates of flow determined at the same infusion pressure are 12.9 cc. in systole and 66 cc. per minute in diastole.   The peripheral coronary systolic
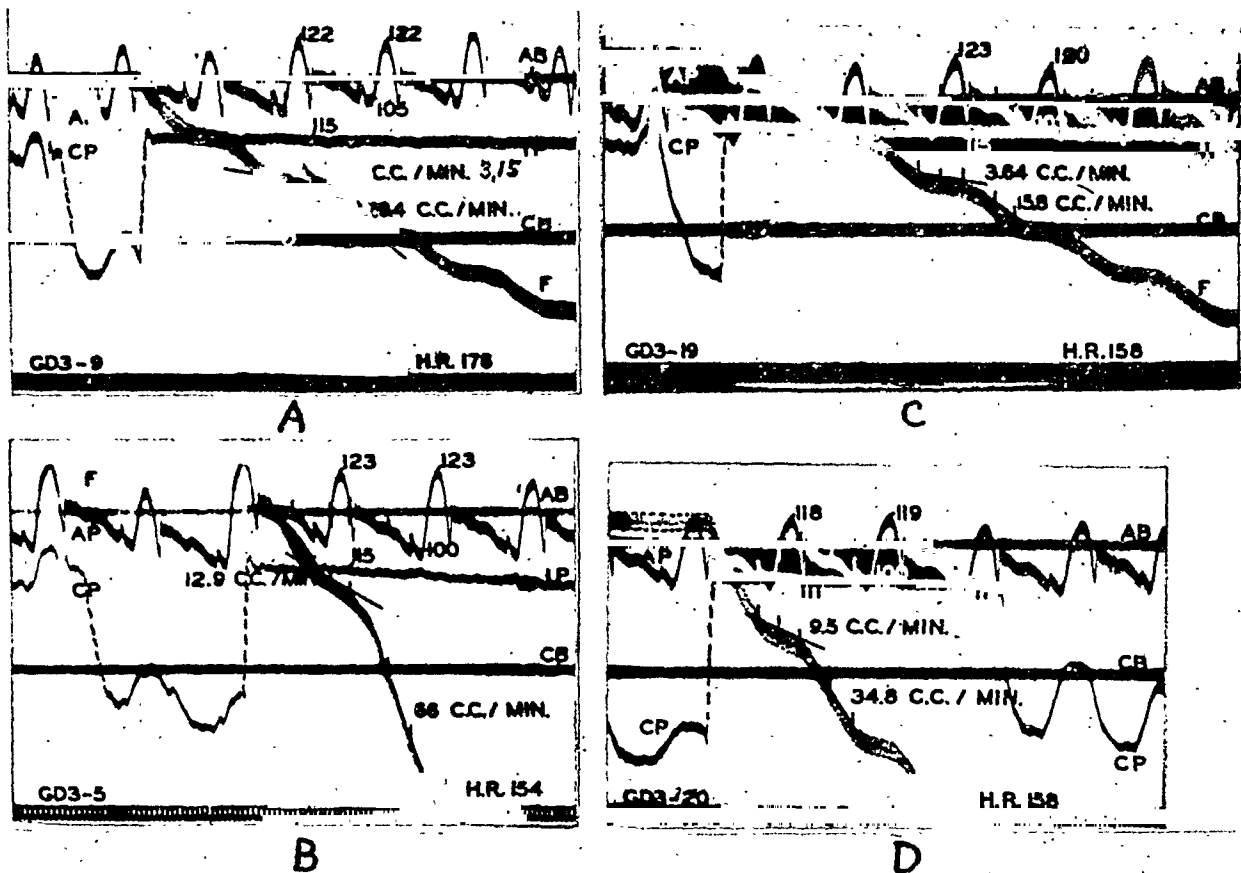
Fig. 1. Records illustrating the effect on coronary blood flow of substitution of Locke's solution for blood (A vs B) and of temporary ischemia of the coronary bed (C vs D).   A, B are the respective controls.   AP, aortic pressure; CP, coronary pressure; AB, CB, aortic and coronary base lines; IP, infusion pressure measured with coronary manometer; F, coronary inflow.

and diastolic pressures are unchanged in the two conditions (85 vs. 87 mm. Hg for systole and 20 vs. 23 mm. Hg for diastole).   Hence with the same differential pressure existing during the two systoles (30 vs. 28 mm. Hg) and diastoles (95 vs. 92 mm. Hg) the corresponding flows are 400 and 300 per cent of the control values.

In other experiments in which the perfusion pressure approximates either the systolic or diastolic peripheral coronary pressure the rate of flow of Locke's, like the controls with blood, approaches zero.

Following temporary ischemia of the coronary bed[2] the inflow is increased greatly without significant alterations of the peripheral coronary pressure. A typical set of records illustrating this point is presented in figure 1C, D. In the control, C, with a peripheral coronary pressure of 90 mm. Hg systolic and 25 mm. Hg diastolic and an infusion pressure of 115 mm. Hg the rates of flow are 3.6 and 15.8 cc. per minute during systole and diastole respectively. After a two minute interruption of the blood supply to the coronary artery the aortic pressure is but slightly lowered and the peripheral coronary pressure is unchanged (D). Despite this the systolic and diastolic flows are increased to 9.5 and 34.8 cc. per minute respectively.

TABLE 1

FIGURE NUMBER	H.R.	AORTIC PRESSURE		INFUSION PRESSURE	PERIPHERAL CORONARY PRESSURE		DIFFERENTIAL PRESSURE		FLOW, CC. PER MIN.		FLOW / DIFF. PRESSURE		CONDITION
		Syst.	Diast.		Syst.	Diast.	Syst.	Diast.	Syst.	Diast.	Syst.	Diast.	
1A	176	122	105	115	85	20	30	95	3.15	19.4	0.10	0.10	Normal
1B	154	123	100	115	87	23	28	92	12.9	66.0	0.46	0.73	Substitute Locke's for blood
1C	158	123	103	115	90	25	35	90	3.6	15.8	0.14	0.18	Normal
1D	158	119	100	115	90	25	25	90	9.5	34.8	0.38	0.37	Ischemia
2A	172	122	105	116	87	20	29	96	2.94	13.97	1.01	1.45	Normal
2B	156	128	110	116	85	20	31	96	2.95	20.10	0.95	2.09	Increased cardiac output
2C	98	70	41	69	53	18	16	51	4.3	44.5	0.27	0.87	Normal
2D	98	135	90	135	89	35	40	94	27.4	108.0	0.69	1.15	Increased aortic pressure

Increased cardiac output augments total coronary flow during diastole while during systole the effect is somewhat variable. The results of the intravenous infusion of blood on coronary flow are reproduced in figure 2 (B as compared with A). Following slow infusion into the jugular vein the aortic pressure is moderately increased and both the systolic and diastolic values of the peripheral coronary pressure are slightly elevated. Perfusion of the coronary bed with blood at a pressure somewhat less than aortic systolic (115 mm. Hg) during both the control period (A) and that of augmented output (B) increases by approximately 40 per cent the diastolic flow, while the systolic flow is unchanged.

When the aortic pressure is raised by compression of the aorta the pe-

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[2] The period of ischemia lasted in different experiments from 30 seconds to 2 minutes, an interval of time sufficiently long that it may be safely assumed that the muscle in the perfused area was extending instead of shortening during systole (5).
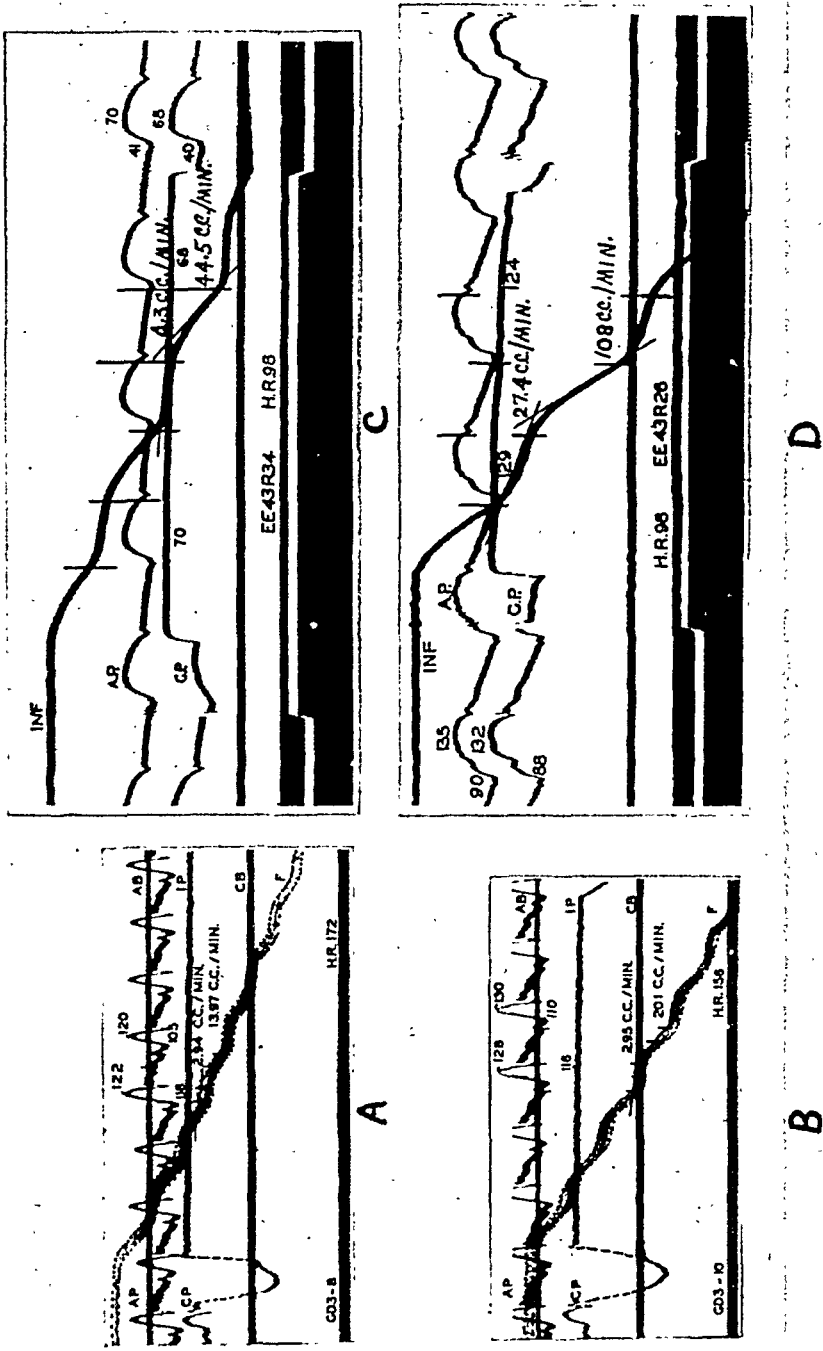
Fig. 2. Records illustrating the effects of intravenous blood infusion (A vs B) and of elevation of aortic blood pressure (C vs D) on coronary inflow. INF, coronary inflow. Other letters same as in fig. 1.

ripheral coronary systolic and diastolic pressures are raised but not as much as the corresponding aortic pressures. The result is a marked increase in both systolic and diastolic differential pressures. This confirms a previous investigation (6). If now the coronary bed is perfused with blood under a pressure corresponding to the aortic systolic the flow is increased throughout the cardiac cycle (fig. 1C, D).

Such comparisons of coronary flow and differential pressures show as observed previously (4) that in the same cycle the systolic flow is generally less than the diastolic for the same differential pressure (cf. table 1). In comparing systoles and diastoles in different circulatory conditions, the flows may or may not change in the same direction as the differential pressures. Following elevation of aortic pressure the flows and differential pressures both increase while in the other conditions listed here the flows all increase but the differential pressures either decrease or undergo no significant change. In addition, the magnitude of the alterations of flow bears no set relationship to the shifts of differential pressure; as a rule the ratio of flow to differential pressure increases greatly (except during systole of increased cardiac output in which the ratio decreases slightly).

SUMMARY AND CONCLUSIONS

The effects of several altered circulatory conditions on coronary flow have been studied during perfusion of the coronary artery with blood under a constant head of pressure. We have confirmed previous findings (using the method of differential pressures) that in elevation of blood pressure and increased cardiac output following augmented venous return, the coronary bed receives an increased blood supply because, although the aortic pressure rises, the peripheral coronary pressure fails to rise as much. However the increase of flow which actually occurs is generally somewhat greater than that predicted from the differential pressures.

In addition, the reduction in viscosity of the perfusate by substitution of Locke's solution for blood causes an unexpectedly large increase of flow,—amounting at times to 300–400 per cent of the rate observed with blood. Also, a period of ischemia of the coronary bed greatly increases the flow during the initial period of restored circulation. Such flow augmentations are suggested by the concomitant slight decrease in peripheral coronary pressure, but the method of differential pressures fails to indicate the magnitude of the flow change.

It is concluded that peripheral coronary pressure curves can accurately represent the time relations of the change of flow to the aortic pressure variations but do not indicate the magnitude of the change in resistance to flow under various circulatory conditions; hence the flow itself is underestimated.

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REGISTRATION AND INTERPRETATION OF NORMAL PHASIC INFLOW INTO A LEFT CORONARY ARTERY BY AN IMPROVED DIFFERENTIAL MANOMETRIC METHOD[1]

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Phasic coronary flow and its determinants have been studied by the method of differential pressures (1, 2, 3, 4) and by the constant pressure flow meter (5, 6). Both are laborious and in addition the former may at times underestimate the extent of variations of flow while the latter does not evaluate the effect of the pulsation of the central coronary pressure.

To obviate these difficulties a method has been devised in which blood from a branch of the aorta is directed through a short external circuit containing a differential meter and then into a coronary artery. This paper is concerned with the use of this meter in registering both the moment to moment rate of inflow and the total inflow into the coronary arteries of dogs under essentially normal conditions.

Apparatus. The meter consists of a device which generates a difference of pressure (roughly proportional to the rate of flow) between two points, and a differential manometer for recording this difference of pressure.

Blood flowing from the aorta *via* the subclavian artery (*SC*, fig. 1) to the coronary artery, *SBC*, passes through a metal tube 2.39 mm. in diameter, approximately 4 cm.[2] long and containing a very thin orifice plate, *D*. The size of the orifice is varied to control the magnitude of the difference of pressure and therefore the sensitivity of the meter. An orifice about 1.27 mm. in diameter was usually employed. Two side tubes (*UT* and *DT*) each 1.19 mm. in diameter open off the main tube. The centers of these are 1.19 mm. from the orifice. To facilitate cleaning and substitution of orifice plates of different sizes the main tube is constructed in two parts which in use are held firmly together by a shell and screw cap (*S* and *S*). In some experiments a Pitot tube arrangement has been substituted for the orifice.

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[1] Preliminary reports of this work were presented before the American Physiological Society at the Toronto Meeting, 1939, and before the Cleveland Section of the Society for Experimental Biology and Medicine, May, 1939.

[2] The actual dimensions may be varied at will but the same proportional relationship should be retained.

When flow occurs, the blood is momentarily accelerated as it passes through the orifice and retains this acceleration for several millimeters downstream. As a result, the velocity of flow past the downstream side tube, DT, is greater and therefore its lateral pressure is less than that of the fluid flowing past the upstream lateral tube, UT. This pressure difference becomes greater the more rapidly the fluid flows.
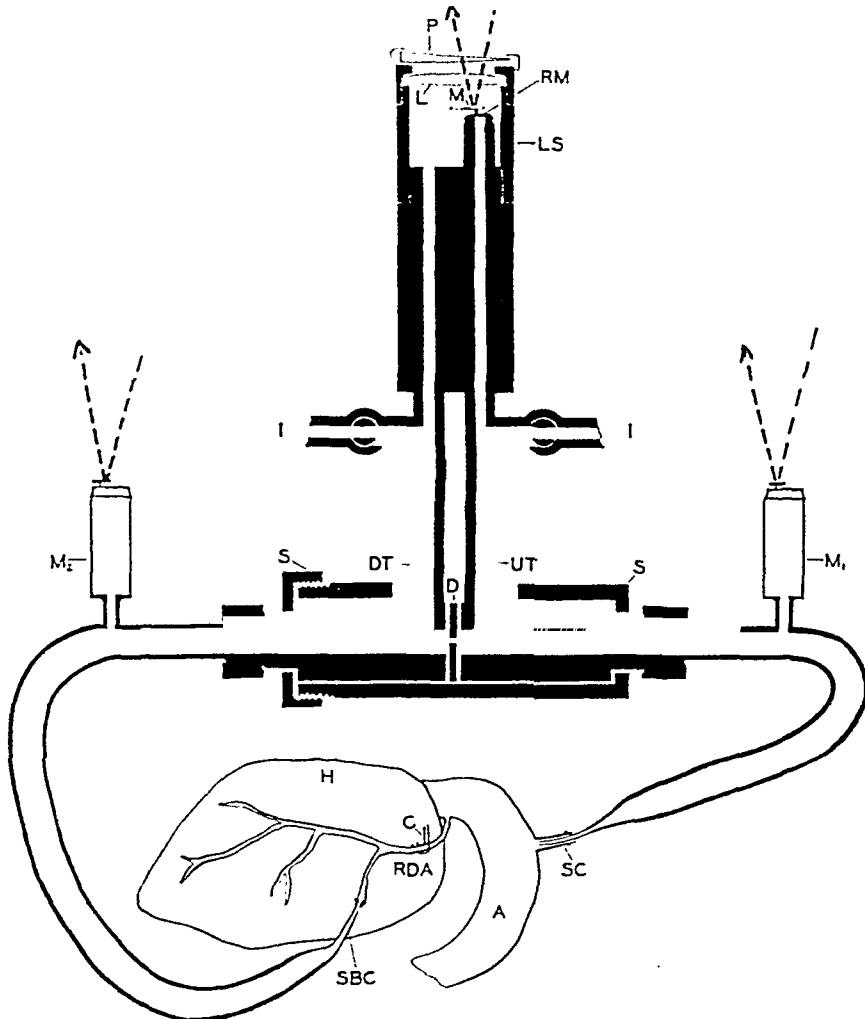
Fig. 1. Diagram of orifice, differential manometer and connections for measuring coronary inflow. See text for discussion and description.

The two lateral pressures are conducted by lead tubes to the differential manometer which is similar in principle to but different in design from that devised by O. Frank (7). The upstream lateral pressure is transmitted to one side of a special rubber membrane, RM, 0.003 to 0.006 inch thick stretched 3–5 times over a manometer tip 4 mm. in diameter. The downstream lateral pressure is transmitted to the other side of this membrane by means of a water-tight chamber, LS, which surrounds the ma-

nometer tip. This is constructed of Lucite to aid in removing air bubbles. A plane mirror, M, (made from a small piece of a Bureau of Standards certified counting chamber coverslip) is coated on its silvered surface and sides with a special adhesive in order to prevent separation of the silver when the mirror is immersed. To make hysteresis minimal the mirror is mounted on the membrane by means of a tiny hard rubber peg and adhesive. Light enters the chamber and is reflected back through a $+0.5D$ planoconvex lens, L, made at least 1.5 mm. thick to prevent warping. Since it is frequently impossible to mount the mirror parallel with the lens, the Locke's solution, with which the chamber is filled, forms a prism which separates the color components of the projected light. To correct this a small angled prism, P, capable of rotation through 360° is mounted in front of the lens. The differential manometer is mounted in the carriage of a Gregg manometer (9, 10). The side tubes, I, serve for filling and flushing the manometer. We have not found it necessary to separate the chamber from the blood by an extra rubber membrane (see 7, 8). The aortic pressure is recorded simultaneously with the flow by means of a Gregg pressure manometer (9, 10). As in the case of the flow meter a combination of a flat mirror mounted on the rubber membrane and a planoconvex lens of proper dioptre reflects the light beam to the camera.

Critique of apparatus. Because of eddy currents induced by the orifice plate, 50 per cent or more of the differential pressure head is permanently lost. To minimize this loss the membrane of the differential manometer is made very sensitive so that a large orifice and therefore small differential pressure can be used. To test the actual loss of pressure in an experiment two pressure manometers (M_1 and M_2, fig. 1) are usually connected to the blood stream some distance above and below the meter. In actual practice the pressure loss may reach 4-6 mm. Hg for a flow of 60 to 80 cc. per minute. In figure 2B, obtained from a mechanical setup in which a pulsatile flow was directed through the meter, the loss of head was 5 mm. Hg for a flow of 50 cc. per minute.

When carefully filled so as to avoid air bubbles the assembled meter has a natural frequency between 70 and 120 dv. per second (tested by elevating slightly the pressure on one side of the membrane and then suddenly allowing the pressures on the two sides to become equalized through the orifice connections). Figure 2C is a reproduction of a frequency curve obtained by this method when the sensitivity was 83 mm. deflection for a 10 mm. change of pressure with the camera at 4 meters.

Calibration. During every set of records a short segment of zero flow is recorded to detect any shift of the relationship between the flow and base line beams. At periodic intervals a complete calibration is made by disconnecting the orifice from the coronary circuit and driving Locke's solution or blood through it at measured rates of flow while recording the deflection of the flow beam.

The accuracy of such calibrations has been tested in two ways (see fig. 3). 1. The deflection of the beam from the zero position is plotted on log paper against the rate of flow at each of a series of flows. In most
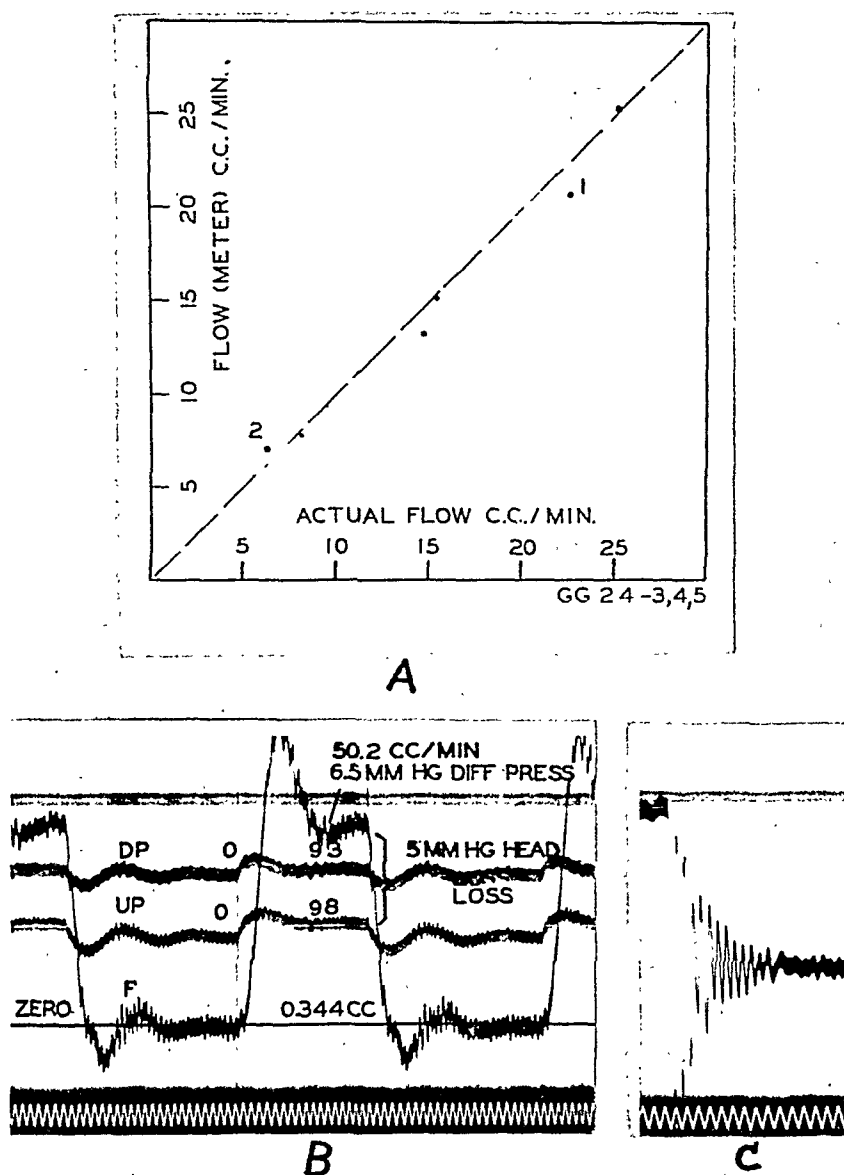
Fig. 2. A, comparison of actual flows with those calculated from flow records. B, example of the type of flow curve used in preparing A. The irregular contour was purposely obtained by adjusting the action of the mechanically operated stopcock which interrupted the inflow to the meter. C, typical frequency curve of the assembled differential manometer and orifice.

instances the points lie along a straight line with a slope of 2:1 indicating that the deflection varies as the square of the flow. 2. Using a high perfusion pressure the rate of flow is controlled by a valve placed first on the upstream and then on the downstream side of the meter, i.e., with first

a low and then a high potential head of pressure in the orifice. In each case the points plot along the same straight line. Changing either the sensitivity of the differential manometer membrane or the size of the orifice displaces the plot but does not alter its slope. When blood is used as the measuring fluid the points fall along the same straight line as those yielded by Locke's solution.

Interpretation of records. The rate of flow at any instant in a recorded curve can be determined by measuring the deflection of the flow beam
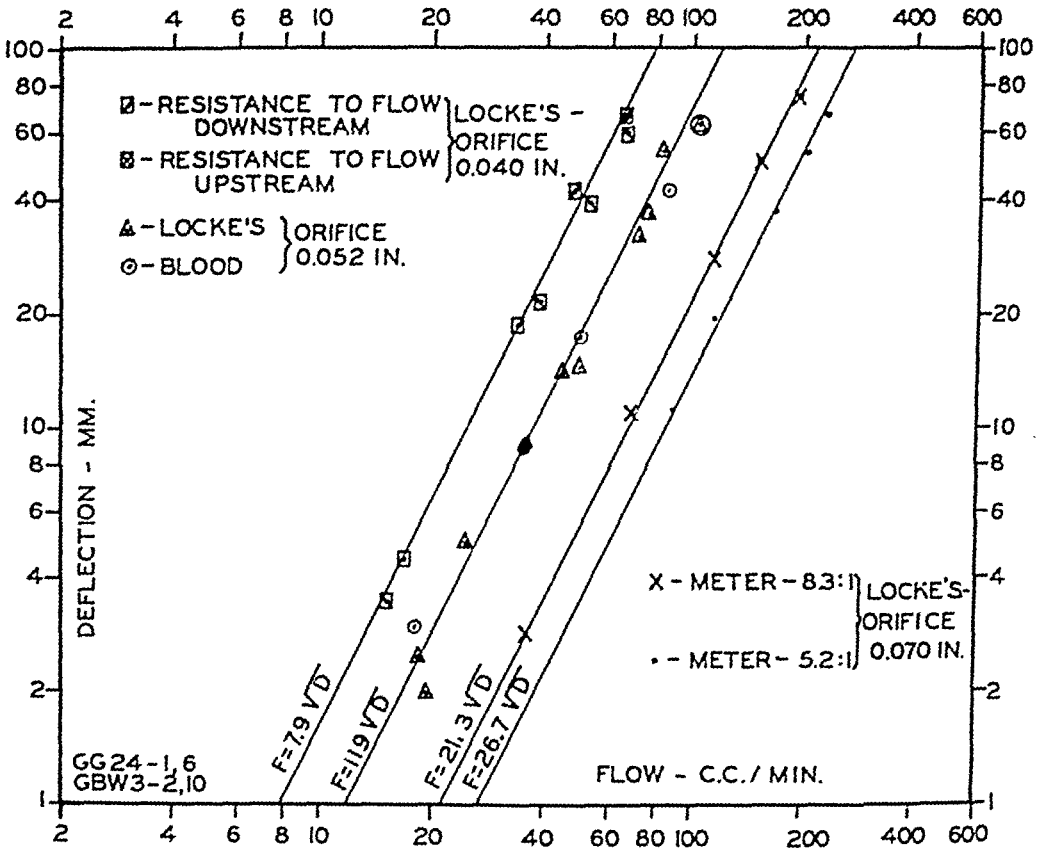
Fig. 3. Tracing of a plot of several flow calibrations on log log paper. Rate of flow (abscissa) in cubic centimeters per minute; deflection of flow beam from the zero position (ordinate) in millimeters. See plot for legend. Discussion in text.

from the zero position and reading off the flow from the plot of the calibration. To determine the total flow during an interval of time it is necessary to determine the average rate of flow for that interval. This may be done conveniently by dividing the area bounded by the flow curve, the zero position and the desired time ordinates by the horizontal distance in millimeters. However, since the deflection of the recorded flow curve varies as the square of the rate of flow, the curve first must be corrected to a linear ordinate scale. This may be done by replotting the ordinate

values of successive points on the recorded flow curve against a linear scale. The curves may be corrected more expeditiously by the use of a square root extractor such as that devised by Broemser (11) and Green (12).

To test the accuracy of the calibration for pulsatile flow the meter was perfused by a stream of fluid under constant pressure which was periodically interrupted by a mechanically operated stopcock. The frequency and speed of interruption, and the rate of flow were varied over wide limits in order to give a variety of contours to the resulting flow curve. Records (see fig. 2B for specimen) were made of the deflections of the flow beam together with collection in a graduate of the fluid flowing through the meter. The total flow for the experimental period was calculated from the recorded flow curve and compared with that which was directly measured. Figure 2A is a plot of several such comparisons. The agreement is good.

Technical procedures. Successful experiments were performed on 17 dogs. The animals were anesthetized with morphine and sodium barbital or sodium pentobarbital. Artificial respiration was begun, the heart was exposed, the main left descending coronary ramus and an adjacent side branch suitable for cannulation were dissected free. After rendering the animal's blood noncoagulable by the intravenous injection of heparin 75 units per kgm. plus chlorazol fast pink 80 mgm. per kgm., the subclavian artery and the side branch of the left coronary artery were cannulated and connected to the meter and manometers. An electrically operated clamp was placed around the central coronary vessel as shown in figure 1. Closing stopcocks (not shown in fig. 1) between the manometers (*M1* and *M2*) and the meter caused the manometers to record the aortic and peripheral coronary pressures respectively and thus gave us the differential pressure records previously described (1, 2, 3) for comparison with the flow records.

RESULTS. *A normal flow curve.* Figure 4, segment 1, reproduces in the lowest curve a normal flow record obtained from the ramus descendens anterior, in the middle curve the downstream pressure, and in the upper curve the upstream pressure. Slightly sloping lines indicate simultaneous points in the curves. At the end of the first diastole, *A*, blood is flowing into the coronary artery at a rate of 28 cc. per minute. Approximately at the onset of isometric contraction, *A*, the rate of flow abruptly begins to diminish, and soon the flow line passes below the zero line, the maximum backflow being at the rate of 7.5 cc. per minute. With the onset of ejection from the ventricle and the rise of aortic pressure, *B*, the backflow diminishes and is rapidly converted to forward flow which reaches a maximum (40 cc. per minute) shortly before the peak of the aortic pressure curve. It then again declines leveling off during the latter part of systole at approximately 20 cc. per minute. Coincident with the closure of the

aortic valve and onset of isometric relaxation, D, the inflow again rapidly
augments (within 0.04 sec.) to 40 cc. per minute and thereafter gradually
declines with the diastolic fall of aortic blood pressure. As mentioned
earlier, the difference of lateral pressure above and below the meter even
at the moment of most rapid flow (i.e., early in diastole) is quite small
(2 mm. Hg). During the period of backflow the downstream manometer,
—as expected, records a pressure higher than the upstream manometer.

Comparison of the flow curve with the differential pressure curve. In seg-
ment 3 of figure 4 is recorded the contour of the peripheral coronary pressure
curve, together with the diastolic value (21 mm. Hg), while in segment
2 the systolic value is determined as 89 mm. Hg.[3] Figure 5A demon-
strates the construction of the corrected peripheral coronary pressure
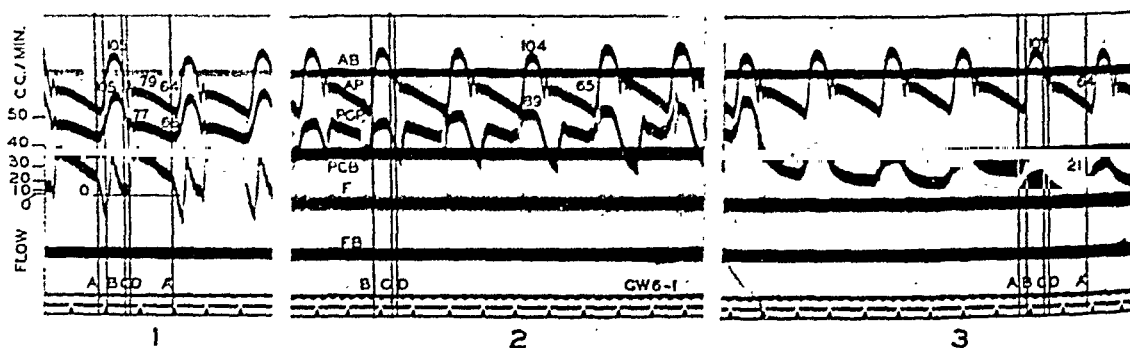
Fig. 4. 1, a typical flow curve. 2, determination of the systolic peripheral coro-
nary pressure. 3, determination of the contour and diastolic value of the peripheral
coronary pressure curve. *AB*, aortic pressure base line; *AP*, aortic pressure curve;
PCP, peripheral coronary pressure curve; *PCB*, peripheral coronary pressure base
line; *F*, flow curve; *FB*, flow base line. Figures along aortic and coronary curves
—pressure values for systole and diastole in millimeters of mercury. Calibration
at left—rate of flow in cubic centimeters per minute—read to top of line. For other
details see text.

curve, *PCP*, from the recorded peripheral coronary pressure curve (dotted
line, traced from next to last cycle of figure 4, segment 3). *DP* is the dif-
ferential pressure curve obtained by arithmetic subtraction of curve
PCP from the aortic pressure curve *AP*.

Segment B, figure 5 (dotted line), is a trace of the recorded flow curve
in figure 4A. *F* (solid line) is the same curve corrected to a linear ordinate
scale. For comparison this curve and the differential pressure curve
of segment A have been put together in segment C, figure 5, by so adjust-
ing their ordinate scales that they coincide at the zero points and at the
rate of flow recorded at the end of diastole. The shaded areas represent
the differences between the two curves. From the standpoint of the

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[3] See the previous papers (8) for discussion of the method.

recorded flow curve the important differences are: 1, a more marked deceleration and the occurrence of actual backflow during isometric contraction
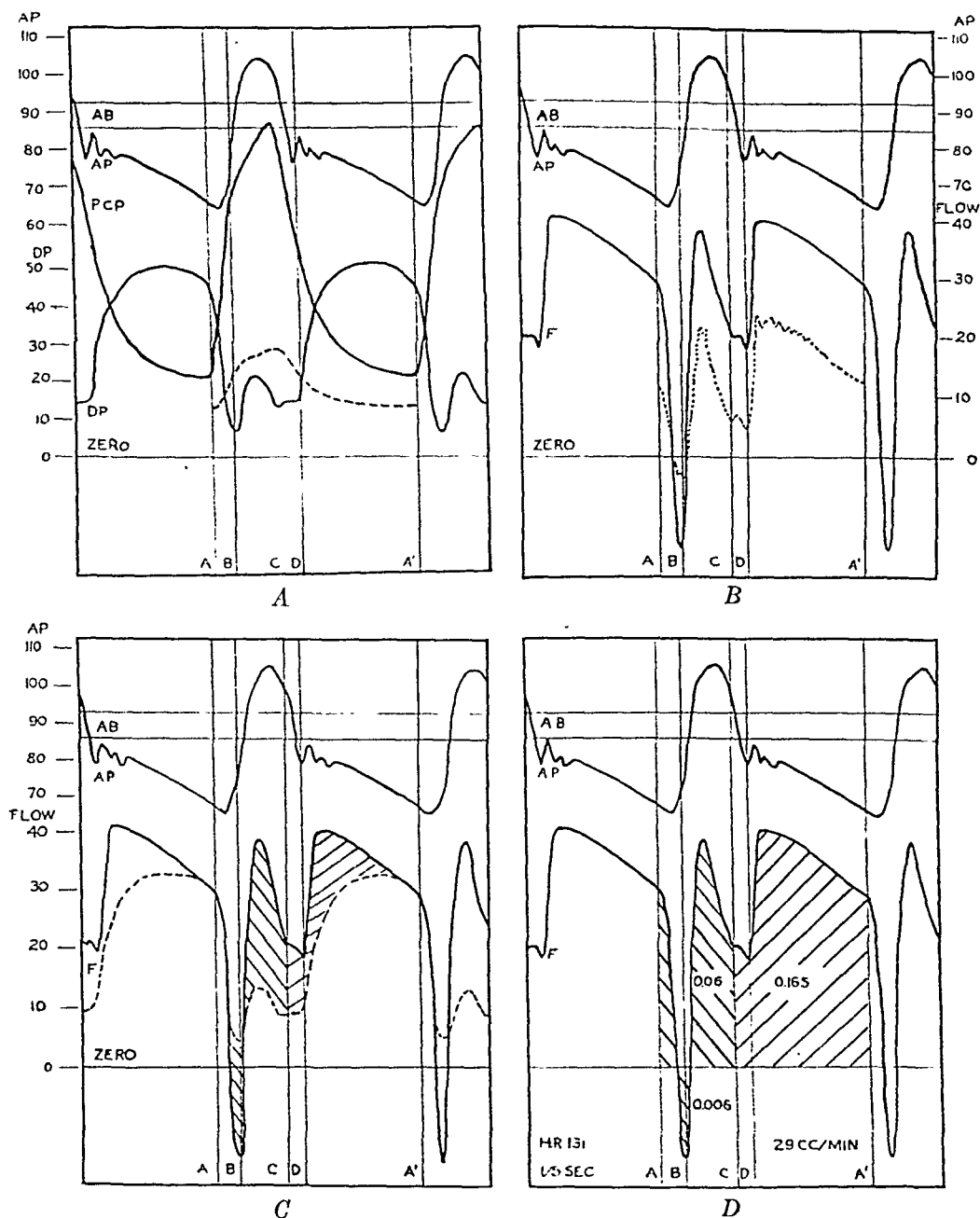
Fig. 5. Diagrams: *A*, reconstruction of a peripheral coronary pressure curve. *B*, tracing of flow curve, dotted line; reconstructed so as to have linear ordinate values, solid line, *F* (compare scale at left with that in fig. 4).   *C*, comparison of differential pressure curve with flow meter curve.   *D*, method of measuring area under the flow curve to get total flow. See text for details.

(A–B), and 2, a greater forward flow during the rest of systole, during isometric relaxation and during early diastole.

The distribution of the total flow. Measurement of the area under the recorded flow curve (segment D, fig. 5) gives a total forward flow during systole of 0.06 cc. and a backflow of 0.006 cc. or a net flow of 0.054 cc. The total flow during diastole is 0.165 cc. or about three times that during systole. With a heart rate of 131 the total cyclic flow is 29 cc. per minute. In a series of experiments on different animals the "normal" systole: diastole ratio of flow varied from 1:1.75 to 1:7.4 with an average figure of approximately 1:2.4. Total flows ranged from 5.7 to 35 cc. per minute with an average figure of approximately 21 cc. per minute.

DISCUSSION. While the flow curve in figure 4 represents the normal inflow into a coronary artery, the use of the moment to moment rate of inflow to interpret the mechanisms affecting flow requires detailed analysis. During a considerable portion of the cardiac cycle the rate of inflow and of intramural flow must differ. The main factors controlling intramural coronary flow are probably: 1, the aortic head of pressure; 2, the resistance to flow, which in turn is dependent upon the degree of contraction of the intrinsic muscles of the coronary vessels and the extent of extravascular compression or support. The factors causing the total rate of inflow to be greater or less than the intramural flow are: 1, the compressor action of ventricular systole on coronary vessels, and 2, the volume elastic effects produced by the pulsating aortic pressure. With these five factors in mind it will be our purpose to see how far the flow curves presented here can be interpreted.

The picture we conceive is as follows: During isometric contraction and early ejection the blood in the deeper lying and more strongly compressed coronary vessels is forced backward into the larger proximal channels and by thus contributing to the supply of blood available for the less strongly compressed and more superficial vessels reduces the inflow from the aorta. This fact is demonstrated by the rise of coronary peripheral pressure and by the backflow recorded by the constant pressure flow meter at appropriate perfusion pressures during isometric contraction and early ejection (5). As the aortic pressure rises, the extramural flow increases due to the increased distention and therefore greater capacity of the more superficial vessels. This effect also ceases at the peak of aortic pressure or slightly later, due to the inertia of the moving column of blood.

During isometric relaxation and early diastole the compressed myocardial vessels are rapidly released, thus causing the total inflow to exceed the actual intramural flow. As the aortic pressure drops, the expansion of the superficial coronary vessels is slowly reduced, thus decreasing the total rate of inflow below the intramural flow. At the end of diastole both effects approach zero.

The magnitude of these effects is large enough to produce serious

alterations of the inflow curve. Constant flow meter studies (5, 6) have shown that ventricular contraction and relaxation may reduce the inflow during systole by 50 per cent and augment early diastolic flow by 25 to 50 per cent. Volume elastic studies made on the coronary vessels (3) indicate similarly that the change in capacity of the vessels as a result of the cyclic change of aortic pressure may reach 25 or 50 per cent of the systolic flow.

From such an analysis it is deduced that during the last part of systole and diastole respectively the factors relating to extramural flow are largely removed and hence the metered inflow may approximate a true measure of intramural flow. In the actual records of flow (figs. 4, 5) the points probably representing intramural flow would be C or D for systole and A for diastole.

In addition, during these two periods separate estimates can possibly be made of the two important factors controlling intramural flow, i.e.,

TABLE 1

NUM-BER	AORTIC PRESSURE		DIFFERENTIAL PRESSURE		*INTRAMURAL FLOW, CC. PER MINUTE		INTRAMURAL FLOW / DIFFERENTIAL PRESSURE		AORTIC PRESSURE / INTRAMURAL FLOW		CONDITION
	Syst.	Diast.	Syst.	Diast.	Syst.	Diast.	Syst.	Diast.	Syst.	Diast.	
1	119	86	12	71	9	22	0.75	0.31	12.1	3.9	Normal
2	140	100	22	85	15	31	0.68	0.37	9.3	3.2	Normal
3	96	70	24	56	26	37	1.10	0.66	3.7	1.9	Normal
4	135	75	43	56	32	37	0.74	0.66	4.2	2.0	Normal

* Flows and differential pressures determined for systole at (C) and for diastole at (A).

the state of contraction of the coronary vessels and their extravascular support. This is possible because at the end of diastole, extravascular compression is at a minimum, while at the height of intraventricular pressure, compression is at a maximum. Comparison of the intramural flow at the latter time with that late in diastole gives a qualitative estimate of systolic extravascular contribution to the control of coronary flow.

Since the intramural flow was found proportional to the differential pressure (5) it is to be expected that with the reservations outlined above the flow curve given by the orifice plate meter should be patterned after the differential pressure curve. Figure 5, segment C, shows that the agreement is good, especially as to direction of movement and time relation of the changes. The differences in amplitude emphasized by the shaded areas approximate in magnitude and agree in direction with those predicted in the above discussion. However, one very definite difference appears; the ratio of flow to differential pressure at the end of systole, i.e.,

at that portion of the cycle when it presumably indicates intramural flow, is greater than the same ratio at the corresponding interval at the end of diastole (compare the amplitudes of curves F and DP at the points indicated by the simultaneous ordinates C and A or A^1 in segment C of figure 5). As evident from representative data in table 1 this is the usual occurrence in different experiments. The true reason for this difference has not yet been ascertained.

Table 1 also shows the ratio of the aortic pressure to the simultaneously recorded rate of flow in cubic centimeters per minute for the same points in the cycle. Comparison of these two shows that the onset of systole increases the peripheral resistance by 2 to 4 times that present in diastole.

SUMMARY

1. A method is described for continuous optical registration of the instantaneous rate of inflow into a coronary artery. This involves shunting the blood from the aorta to the coronary artery through a short external circuit containing an orifice (or other device) connected with a differential manometer.

2. The left coronary inflow curves so obtained demonstrate that beginning approximately at the onset of isometric contraction there is a rapid retardation of flow but that with the rise of aortic pressure during ejection the inflow rapidly accelerates, reaching a peak during the middle of the rise of aortic pressure and then declining to a more or less constant rate of inflow during the latter part of systole. Following the incisura there is again a rapid acceleration, the inflow reaching a peak early in diastole and then declining with the progressive fall of aortic pressure in diastole.

3. The inflow records are complicated by volume elastic effects due to the cyclic rise and fall of aortic pressure and by a compressor action of ventricular systole.

4. Despite these complications, and unless some other unknown factors are operating, it seems probable that the rate of inflow at the end of diastole just preceding isometric contraction, can be used as an index of intramural flow during diastole.

5. Similarly it seems probable that the rate of inflow during the brief interval at or just preceding the onset of protodiastole, i.e., at the peak of the peripheral coronary pressure curve, can be used as an index of the systolic rate of intramural flow. In almost all instances the systolic intramural flow so measured is of sizable magnitude.

6. The rate of intramural flow per millimeter of differential pressure is greater during systole than during diastole.

7. Simultaneous measurement of aortic pressure and rate of intramural flow indicates that the resistance to blood flow existing during the latter part of diastole is increased from 2- to 4-fold during systole.

8. The total flow may be determined from the moment to moment flow curve by appropriate procedures.

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CHANGES IN THE CORONARY CIRCULATION FOLLOWING INCREASED AORTIC PRESSURE, AUGMENTED CARDIAC OUTPUT, ISCHEMIA AND VALVE LESIONS[1]

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In previous work (1, 2) the manner in which the relative systolic and diastolic flows through the left coronary are modified under various dynamic conditions, normal and abnormal, were estimated by analysis of differential pressure curves taken from a coronary ramus. Since we have now found with a constant pressure meter (3, 4) that such pressure differentials can alter less than the flow, we have repeated these experiments registering simultaneously pressure differentials and inflow by means of the orifice meter described in the preceding communication (5).

The results of typical experiments are grouped together in figure 1 and the essential data taken from each of these curves are given in table 1 under corresponding letters.

Elevation of aortic pressure. Blood pressure was elevated by compression of the thoracic aorta. As shown by comparison of records in figure 1, A and B, the systolic and diastolic flows increase significantly. The minute flow, i.e., the sum of the systolic and diastolic flows times the heart rate, increases from 10.3 to 36.4 cc. per minute during the increase in mean pressure from 22 to 91 mm. Hg. This confirms previous work on differential pressures (1). However, the increases are proportionately much less than the elevation of aortic blood pressure. If the ordinate lines A and B are regarded as indicating the times when coronary flow is largely intramural (5) both systolic and diastolic intramural flows are augmented, the former from 0 to 24 cc. per minute and the latter from 14 to 43 cc. per minute. However, as shown in table 1, A, B, the systolic flow increases more than and the diastolic less than the corresponding differential pressures.

Increased cardiac output. (C and D of fig. 1 and table 1.) When blood warmed to body temperature is slowly infused into the jugular vein the

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Fig. 1. Records showing the effects on coronary blood flow of aortic compression (B), blood transfusion (D), ischemia of the vascular bed (F), and aortic insufficiency (H). Respective controls are A, C, E, G. Vertical lines (A, B) indicate approximate ends of diastole and systole. AP, aortic pressure, CP, coronary pressure, CF, coronary flow. Time $\frac{1}{5}$ second. Calibration accompanying each pair of records = rate of flow in cc./min.
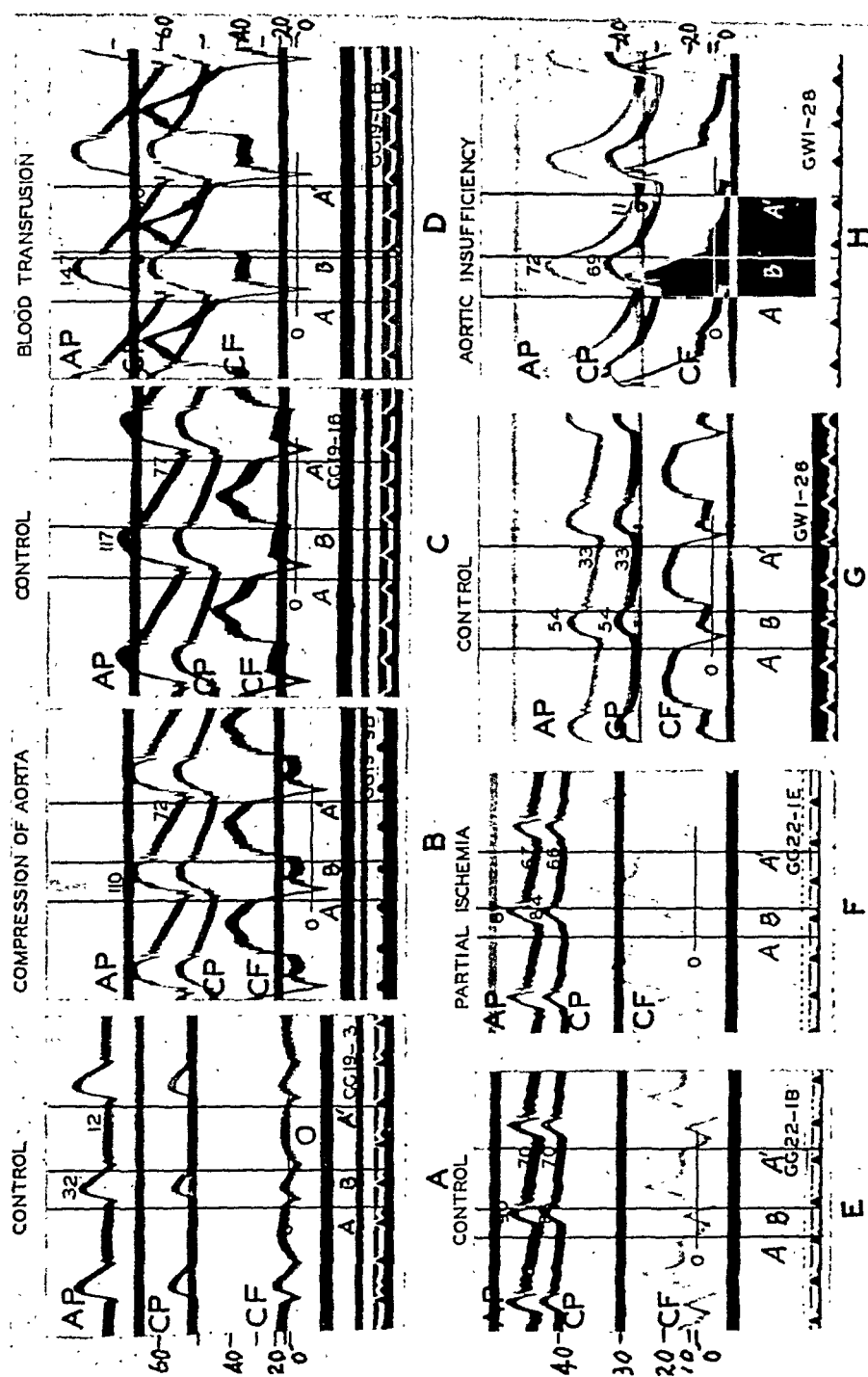
minute coronary flow increases from 30 to 49 cc. per minute. Only a slight increase of peripheral coronary pressure occurs despite the considerable elevation of aortic pressure and as a result the differential pressure increases considerably both in systole and diastole. The increase in systolic flow recorded by the meter is approximately proportional to the increase of differential pressure but in diastole the flow increases more than the differential pressure.

Ischemia of the myocardium. In the experiment illustrated in figure 1 and table 1, E and F, a coronary ramus was occluded for about 30 seconds. Upon restoration of the blood flow the total and intramural flows showed large increases both during systole and diastole, while the aortic pressure and peripheral coronary systolic and diastolic pressures decreased con-

TABLE 1

RECORD NUMBER	HEART RATE	AORTIC PRESSURE		AORTIC PRESSURE AT "B"	PERIPHERAL CORONARY PRESSURE		DIFFERENTIAL PRESSURE		INTRAMURAL FLOW, CC. PER MINUTE		TOTAL FLOW, CC.		INTRAMURAL FLOW / DIFFERENTIAL PRESSURE		CONDITION
		Systole	Diastole		Systole	Diastole	Systole	Diastole	Systole "B"	Diastole "A"	Systole	Diastole	Systole	Diastole	
A	114	32	12	18	25	3	−7	9	0	14	0.0235	0.067	0	1.56	Control
B	115	110	72	108	85	15	23	62	24	43	0.077	0.24	1.04	0.69	Aortic compression
C	90	117	77	115	85	16	30	62	23	36	0.115	0.245	0.77	0.58	Control
D	89	147	90	140	90	16	50	74	40	50	0.16	0.395	0.80	0.68	Blood transfusion
E	131	90	70	80	75	22	5	48	0	15	0.017	0.125	0	0.21	Control
F	139	87	67	80	69	11	11	47	14	27	0.052	0.17	1.28	0.575	Ischemia
G	119	54	33	50	50	10	0	23	12	25	0.047	0.14	∞	1.09	Control
H	110	72	11	70	55	10	15	−1	26	0	0.089	0.56	1.73	0	Aortic insufficiency

siderably. The systolic differential pressure rises but the diastolic remains unchanged. As a result, the flows increased much more than the differential pressures. The minute flow increases from 17.3 to 30.8 cc. per minute.

Aortic insufficiency. We have confirmed a previous observation (2) that in aortic insufficiency the differential pressure increases during systole and decreases during diastole. The metered flows vary in the same direction but change much less proportionately than the pressure differential (G and H of fig. 1 and table 1). In the example shown here due to the extreme fall of diastolic pressure the increase of systolic flow does not compensate adequately for the lower diastolic flow, and hence the minute flow decreases from 22.5 cc. to 16 cc. per minute.

Aortic stenosis. Several observations made before and during this

disturbance indicate that the flow changes correspond closely with those predicted from the differential pressure curves, namely, that there is little effect on diastolic flow but a marked reduction of systolic flow.

SUMMARY AND CONCLUSION

Records of the moment to moment rates of flow and of the total inflow into the left coronary artery of dogs have been taken with the orifice meter together with the aortic and peripheral coronary pressures under different dynamic conditions.

Study of such indicates that during both systole and diastole the total and intramural flows increase following aortic compression, blood transfusion, ischemia and aortic insufficiency (only during systole), while in diastole of the latter the intramural flow decreases and the total flow increases. The pressure differentials follow in the direction of the metered flows but since they change much less they can provide only a qualitative measure of flow. These differential pressure changes may be less than, greater than or the same as the flow alterations.

The latter findings permit certain deductions, provided one subscribes to the idea previously advanced (5) that changes especially during diastole, in the ratio of intramural flow to differential flow may indicate alterations in size of the available coronary bed, and in addition that changes in systolic peripheral coronary pressure reflect changes in extravascular compression or support.

Calculations made upon this basis indicate that following increase of cardiac work through simple elevation of aortic pressure the available coronary bed becomes smaller while in ischemia and in augmented cardiac work due to increased cardiac output the bed increases, because in the former the diastolic flow increases less than the pressure differential while in the latter the reverse is true. Substantiating this is the observation that the minute flow per millimeter Hg aortic pressure decreases with aortic compression and increases with augmented venous return.

In elevation of aortic pressure, augmented cardiac output, aortic stenosis and aortic insufficiency, but not ischemia, the extravascular support is presumably increased as evidenced by the increased peripheral coronary systolic pressure. However, failure of such increase in extravascular compression to rise concomitantly with the aortic systolic pressure, is in part responsible for the augmentation of systolic flow in these conditions and its converse for the reduction of flow in aortic stenosis.

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OBSERVATIONS ON THE GENESIS OF THE ELECTRICAL CURRENTS ESTABLISHED BY INJURY TO THE HEART[1]

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While considerable work has been done on the electrical potentials established by injury to the heart (reviewed by Schütz (1)) and an analysis of the changes so produced in the electrocardiogram has proved fruitful in clinical practice (cf. Bohning et al. (2), for example), certain aspects of the problem are still unsettled. This is particularly true of the genesis of the characteristic T wave deformity that occurs after injury.

In the present report, our observations from two types of experiments will be discussed. In the first the course of electrical events was followed in a spot on the mammalian ventricle during the development of and recovery from an injury produced by compression. In the second, the course of electrical events was followed in a series of approximately equidistant spots within and outside of an injury produced by injection of 95 per cent alcohol.

I. *The course of electrical changes during the production of and recovery from a small injury in the dog's heart.* Two experiments were done with identical results. The animals were anesthetized with nembutal (25 mgm./kilo), the anterior chest wall removed and artificial respiration maintained. The pericardial sac was opened and arranged to cradle the heart. Unipolar leads were used with the indifferent electrode placed beneath the skin of the left leg and the direct electrode on the surface of the right ventricle. Both electrodes were non-polarizable, and each consisted of a wick soaked in saline-agar and fastened to a porcelain boot filled with zinc sulfate solution into which a zinc electrode was placed; the copper lead wire was attached to the zinc electrode. The connection to the electrocardiograph was such that negativity of the cardiac electrode gave an upward deflection in the record. The wick of this electrode was incorporated in a pressure electrode, consisting of a small glass tube, tapered at the end in contact with the heart (previously described, Jochim, Katz and Mayne, 3); the wick was passed through the tube and projected about 0.5 mm. from the tip so as to pick up the electrical currents from

the center of the injury. The records were standardized so that 10 milli-volts gave a deflection of $\frac{1}{2}$ cm.

After placing the electrodes, the one on the heart very gently so as not to cause injury, a continuous record was made. During the course of the recording, gradually increasing pressure of a degree sufficient to cause injury was manually applied on the heart electrode (cf. Jochim, Katz and Mayne, 3), and then later, the pressure was gradually released. This maneuver was repeated several times.

In figure 1 a typical experiment is shown with segments of the record before injury (A), during increasing injury (B), during the injured state (C), during recovery from injury (D), and after recovery had been completed (E). The sequential changes in potential of every second beat in
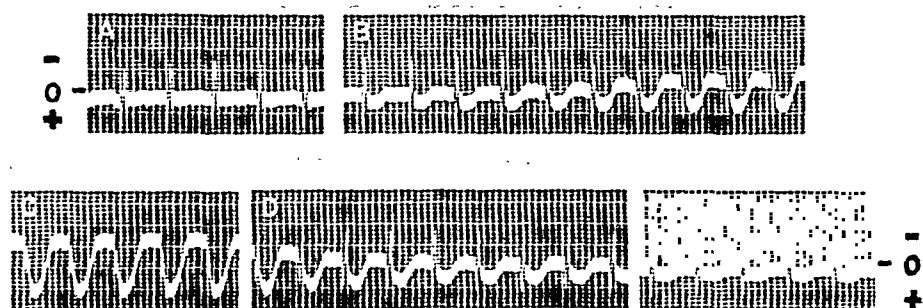
Fig. 1. Segments from a continuous record of a unipolar lead from a spot on the dog's ventricle showing changes occurring during the development of and recovery from a small injury produced by pressure on the special cardiac electrode. A, control before pressure was applied; B, during application of increasing pressure; C, during period of maximum injury; D, during release of pressure; E, after release of pressure, showing almost complete recovery.

Indifferent electrode was on left hind leg. The intervals between successive segments of the record ranged from approximately 2 to 10 seconds. The isoelectric level for the top of the line is marked 0; upward deflection = negativity ($-$) of cardiac electrode, downward, positivity ($+$). Discussed in text.

one experiment where compression was applied and released twice are charted in figure 2. The upper row of dots represents the negative potential of the injured area during diastole of the ventricles at the moment just before the beginning of the QRS complex. The negativity was measured with respect to the potential of this spot on the heart at the same moment before injury, i.e., the potential of the resting uninjured muscle. The lower row of dots represents the maximum positive potential developed at the injured area during ventricular systole, i.e., at the lowest level of the S-T depression; the same reference potential was employed as that used for the upper row of dots. In these experiments it is assumed that the potential of the "indifferent" electrode remains essentially constant (Wilson et al. (4, 5)). Since each millimeter of the record

equalled 2 millivolts the potentials would be twice that of the ordinate scale. Since the cycle length was 0.25 sec., each dot represents 0.50 sec. in time.

Inspection of these two figures will show that this compression type of injury produced the following almost entirely reversible changes in the potentials of the injured area:

1. A decrease in QRS amplitude involving the upright phase more than the inverted one; the persistence of this part of the electrical curve
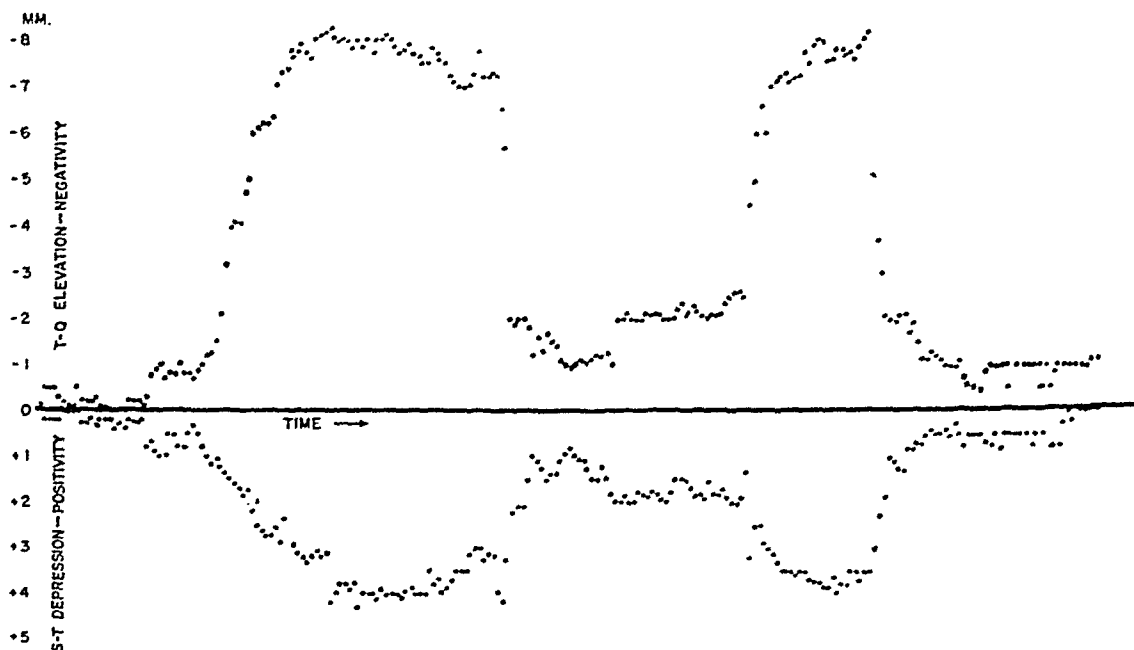
Fig. 2. Chart showing the time course of the development and regression of the T-Q elevation and S-T depression caused by the development of and recovery from injury produced with the pressure electrode. Two periods of compression and decompression are shown. The maximum displacement in millimeters of the T-Q and S-T intervals of every second beat from a continuous record were plotted against time on the abscissa. Since the cycle length was 0.25 sec., the time interval between any two successive points is 0.50 sec. The zero line represents the potential of normal resting muscle, i.e., just before the beginning of the QRS in the control record before compression. Discussed in text.

was responsible for the notching of the downstroke of the full blown monophasic deflection (seg. C., fig. 1).

2. The development of a depression (positivity) of the S-T segment (with the disappearance of the T wave) below the isoelectric line of the curve before injury. Occasionally, a slight residue of this change persisted after the injurious compression on the electrode was relieved (seg. E., fig. 1).

3. The development of an elevation (negativity) of the T-Q interval above the isoelectric line of the control curve before injury.

4. The extent of the S-T and T-Q level shifts were not equal but they followed a similar time pattern of development and disappearance.

Our observations confirm those recently made by Eyster, Meek, Goldberg and Gilson (6) using different methods of producing injury and lend support to their view that *injury causes the development of a potential distribution which can be explained on the assumption of the existence of two concentric rings of charges, the inner one negative and the outer one positive during ventricular diastole, and the reverse polarity during ventricular systole.* We have explored the field in model experiments
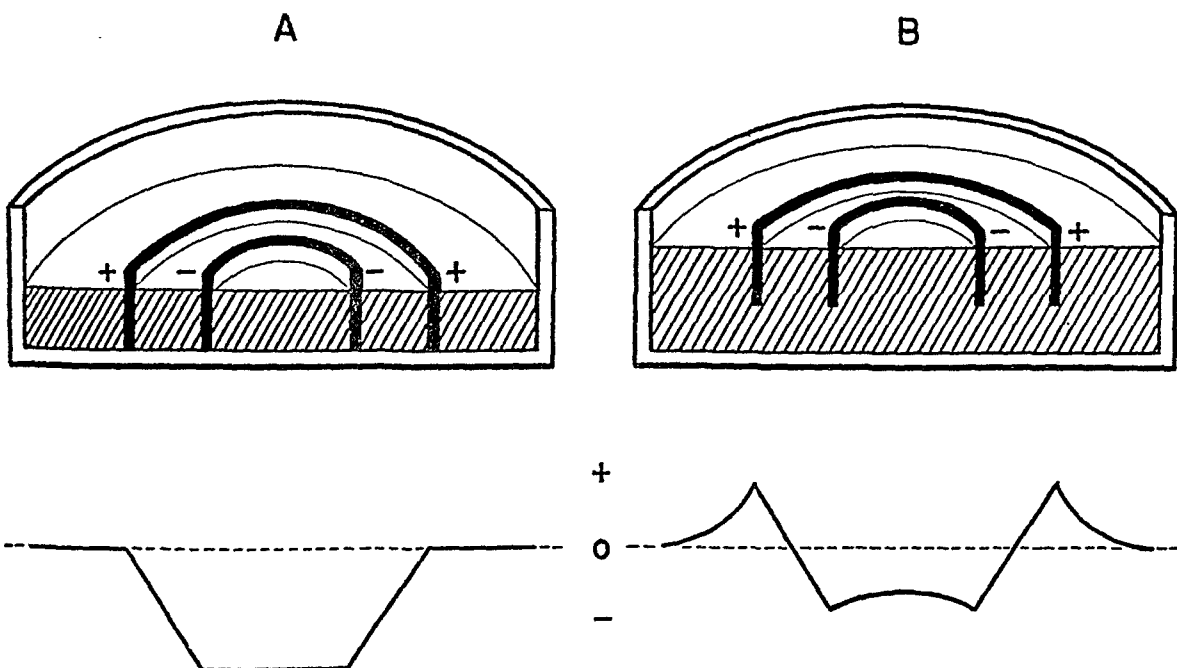
Fig. 3. Diagram showing the potential distribution in a saline field produced by two concentric rings of opposite charge. In A, the rings are fastened to the bottom of the dish so that the saline is divided into 3 separate compartments. This arrangement gives the potential distribution, shown below diagram, calculated by Eyster, Meek et al. (6). In B, the rings are merely immersed below the surface of the saline without breaking its continuity. The resulting potential distribution is shown below the diagram. In each, the zero level represents the potential of a point on the edge of the field. Discussed in text.

with such concentric rings and have confirmed the potential distribution postulated by Eyster, Meek et al (6). It is important to point out that in order to obtain this potential distribution the concentric rings must be so placed in the field that they divide it into three separate regions having no communication with each other except through the rings themselves (fig. 3 A). If the rings are immersed in a saline field without interrupting its continuity, the potential distribution is as shown in figure 3 B, with a positive phase at the outer ring which falls off to zero as the periphery of the field is approached.
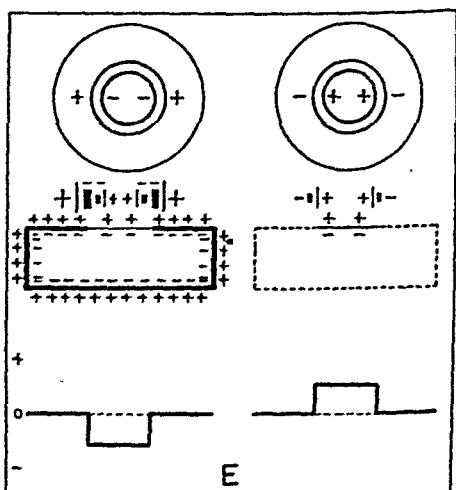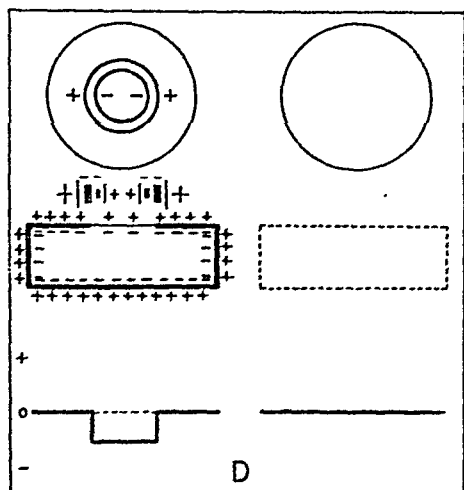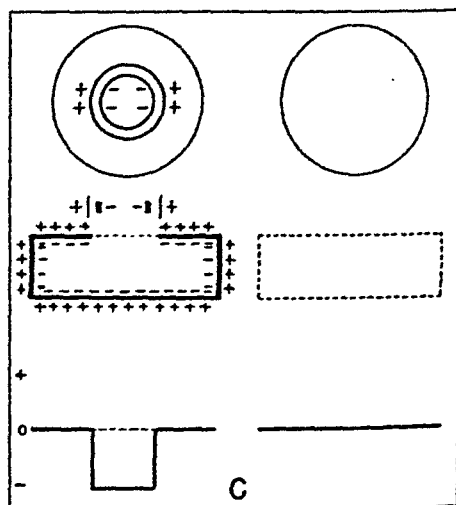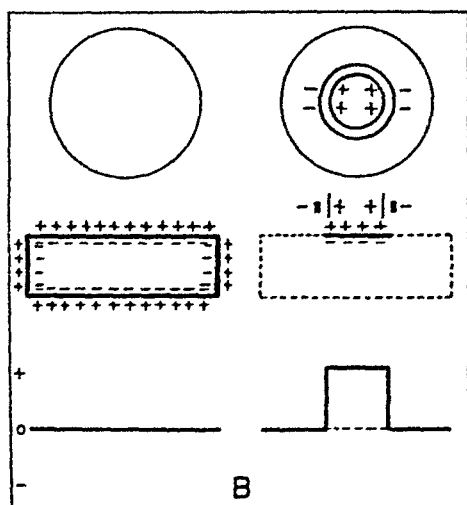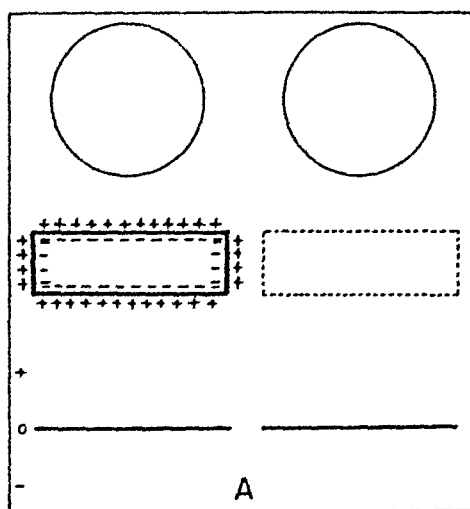
Fig. 4

This electrical solution of the effect of injury is readily related to the classical membrane theory. In figure 4, we have attempted to illustrate this correlation. In A the proposition is presented that at rest the electrical state of the syncytial membrane may be represented by a continuous polarized membrane with negative charges on the inside and positive on the outside; hence the external surface of the cell will be of zero potential during diastole, i.e., it will have the same potential as the indifferent electrode in the external field. During complete activation the polarized state being destroyed, no battery will exist and hence again the cell surface will be of zero potential, i.e., it will have the same potential as it had during rest.

However, several other possibilities exist: 1, a region of normal resting polarity may not respond during systole and remain polarized; 2, a region of complete depolarization may be caused by injury; 3, a region of partial depolarization may exist during diastole which responds during systole and has its depolarization completed, and 4, a region of partial depolarization may exist during diastole which does not respond during systole.

B of figure 4 shows the first of these possibilities. During diastole, this is like a normal cell but during systole the remaining polarized part of the cell will act as the source of current and give rise to a distribution of potential which can be produced by two concentric rings of charges, the outer ring negative and the inner positive. A monophasic action curve during the heart cycle could thus be obtained from this region without any injury current being present at rest. This has been described to occur by Ashman and Woody (7).

C of figure 4 shows the second of these possibilities. The injured area is completely depolarized at rest and hence a unipolar or bipolar lead from this region will show a current of injury, the polarized uninjured part of the cell acting as the current source. The distribution of charges can be represented by two concentric rings, the outer positive, the inner negative. During systole, this double ring will disappear since the entire

Fig. 4. Diagrams representing the distribution of *charges* on the two surfaces of a cell membrane at rest (on the left in each diagram), and during complete activation (on the right). The heavy solid lines of the cell wall represent complete polarization, the thin solid portions represent partial polarization, and the dotted lines indicate complete depolarization. Just above each cell is indicated the resultant battery produced by the assumed distribution of charges. Below each cell is shown the *potential* distribution across the upper surface of the cell, referred to a distant point in the field as zero. Above each cell is shown a circular field viewed from above with appropriate concentric rings of opposite charge to give the potential distribution shown. These rings are assumed to be placed in the field as in figure 3 A (some slight modification would have to be made if the rings were as in 3 B). A, normal cell; B, cell with a region *not responding during activation;* C, cell with a *region of complete injury;* D, cell with a *region partially injured but responsive;* E, cell with a *region partially injured and irresponsive.* Discussed in text.

cell will be depolarized. This state will also give rise to a monophasic curve from this region during the heart cycle.

D of figure 4 represents the third possibility. Here the injured area is only partially depolarized at rest. This is really a variant of the second possibility, the only difference being that the resting current of injury and the monophasic action current will be smaller in magnitude since during diastole the injured area will also act as a current source which will neutralize in part the effect of the uninjured part of the cell. During systole, both sources of current will be eliminated. No reports of the actual occurrence of the foregoing possibilities (C and D) have appeared in the literature.

E of figure 4 represents the fourth possibility. During diastole a current of injury will flow for the same reasons as in cases C and D; but during systole the lack of response of the injured region will cause a reversal of the polarity of the concentric rings since the injured part of the cell will be the only part of the cell which will serve as the current source. This is the type of monophasic curve which Eyster, Meek et al. (6) and we, ourselves, have obtained.

It is apparent from the above description that these newer observations are not contradictory to the classical view. The observations of Eyster, Meek et al. have, however, served to define the classical membrane theory in more precise terms. It has led us to the idea that regions may be injured, irresponsive, or both and while in each case monophasic curves occur, the potential changes will differ in each case.[2]

The concept outlined above to account for the changes in the electrical-time curve is not contradictory to the observations reported previously from this laboratory (Jochim, Katz and Mayne, 3). It is apparent that the bipolar lead from an injured to an uninjured area on the heart is the algebraic sum of the electrical variations from each as has recently been shown experimentally by Eyster, Meek et al (6). The onset of the monophasic curve in the bipolar lead used by us was thus the same point as that used in uninjured curves in unipolar leads (cf. fig. 5) and would, therefore, depend on when the activation in the uninjured area began. The onset and end of the monophasic curve in unipolar leads would be the same for all spots of the heart since as shown above it would be determined by the time at which depolarization begins and repolarization ends in the ventricles. The end of repolarization in the uninjured area is shown in the unipolar lead by the end of the T wave. In bipolar leads, this T wave summating with the curve from the injured area will alter the con-

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[2] The changes in the potential of the QRS in unipolar leads is dependent, in all likelihood, upon the presence of irresponsive areas beneath and adjacent to the electrode causing injury, and the degree of change in QRS is no doubt a rough measure of this irresponsiveness.

tour and time of termination of the monophasic curves.   It is thus apparent that the points used by us to measure the duration of the monophasic curve would be an index of the duration of activity of the uninjured area
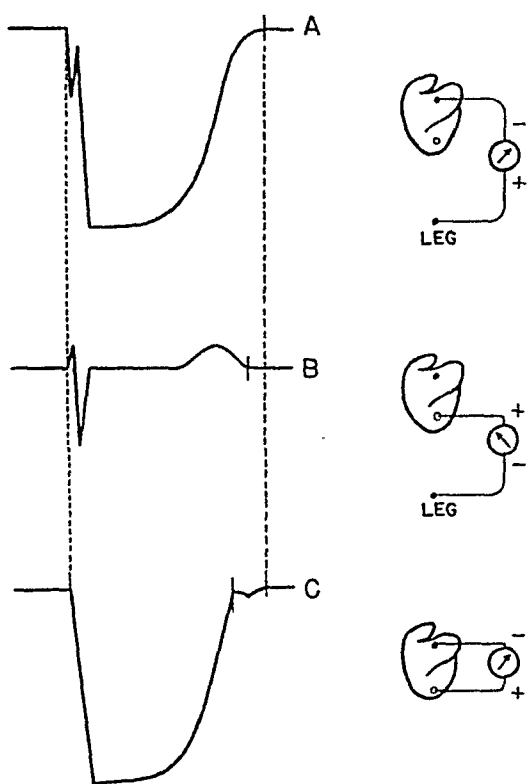
Fig. 5   Fig. 6

Fig. 5. Diagram showing summation of a unipolar lead from an injured area (A) with that from an uninjured area (B) to give the resultant bipolar lead of different duration (C), diagram of connection to galvanometer is shown on the right of each curve. Discussed in text.

Fig. 6. Diagram with conventions as in figure 4 to illustrate the possible state of the cell membrane polarization during the inscription of the coronary type T wave found in the annular region around the injury—resulting from the *temporary lag in repolarization of a partially injured responsive area*. Discussed in text.
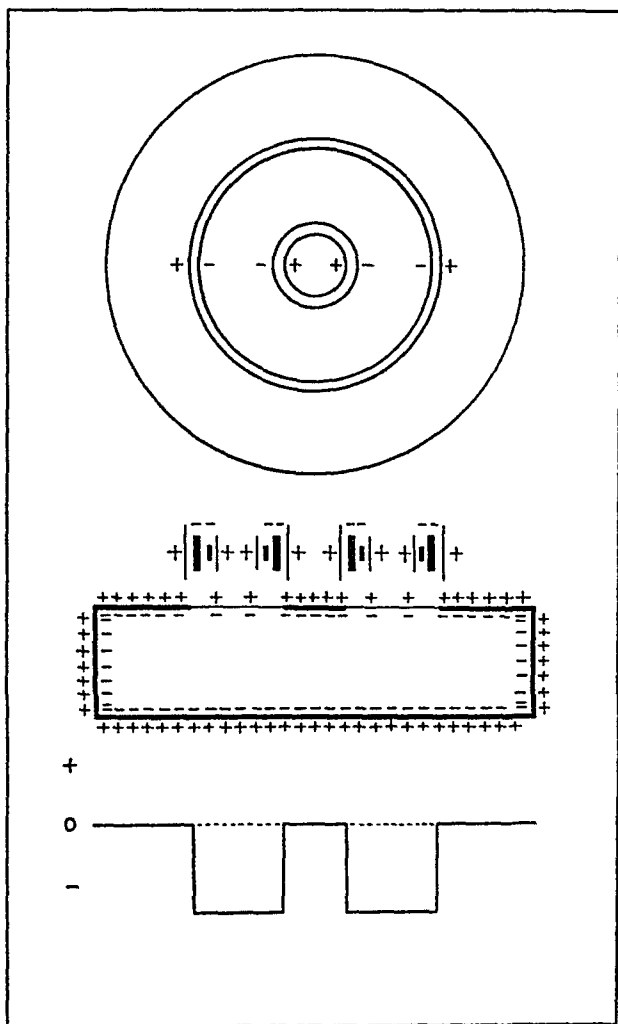
and would vary with the location of the electrode on uninjured but not with that of the electrode on injured regions.   ·   .

II. *The course of electrical events in a series of approximately equidistant spots within and outside of an injury produced by 95 per cent alcohol.* Ex-

periments were done on ten animals anesthetized with nembutal (25 mgm./kilo). The procedure of exposing and preparing the heart was the same as in the preceding experiments. Similar non-polarizable wick electrodes were used, those on the heart not being enclosed in the pressure electrode but, instead, permitted to just rest on the epicardium. These wicks were fastened to their respective spots by very fine epicardial sutures, care being taken to wait for complete recovery from this very mild injury before starting the experiment. Unipolar leads were used, and these were standardized so that 10 millivolts equalled $\frac{1}{2}$ cm. Normal control records were taken from a number of spots spaced equally along a straight line across the surface of the right ventricle. The records were taken in quick succession by connecting each electrode on the heart in turn through the galvanometer with the indifferent electrode by means of a rotary selector switch.

After the control records were taken, a localized area of injury was produced by the intramyocardial injection of 2 cc. of 95 per cent alcohol. This injured area usually extended beneath 2 to 4 of the spots on the heart upon which electrodes had been placed. The remaining spots extended over tissue near the margin of the injury and well out into normal tissue. Immediately after production of the injury, the records were again taken from exactly the same spots that were used before for controls. These were repeated at lengthening intervals until the records showed a return to the normal contour, a period of $1\frac{1}{2}$ to $4\frac{1}{2}$ hours.

The results of a typical experiment are shown in figure 7. In this experiment the analysis was integrated by making a series of 3 dimensional models (cf. fig. 8). Each model represents the time-space distribution of potentials before and at certain times after the production of the injury. For each model, one cycle of the record from a single spot was enlarged 5 fold, a cardboard backing pasted on each record and the curve cut out along its upper border. These cutouts were mounted vertically on a stand, the cycle from each spot 1 cm. behind the other, with the point just before QRS in each in the same horizontal and the same vertical planes. It is recognized, of course, that placing these points on the same horizontal level ignores the shift in potential of these spots as injury is induced, but this is not disturbing for the purposes of our analysis. The space between the cutouts was filled with modelling clay and smoothed over to make a three dimensional time-space-potential diagram. The axes represent: height, voltage; breadth, time in heart cycle; and depth, distance along heart surface. Before photography the models were coated with Alco-glaze and then painted white in order to preserve them and make reproduction easier. The models represent: A, control before injury; B, immediately following injury; C, D, E, F, G, and H, respectively 5, 15, 30, 45, 60 and 75 minutes after injury.
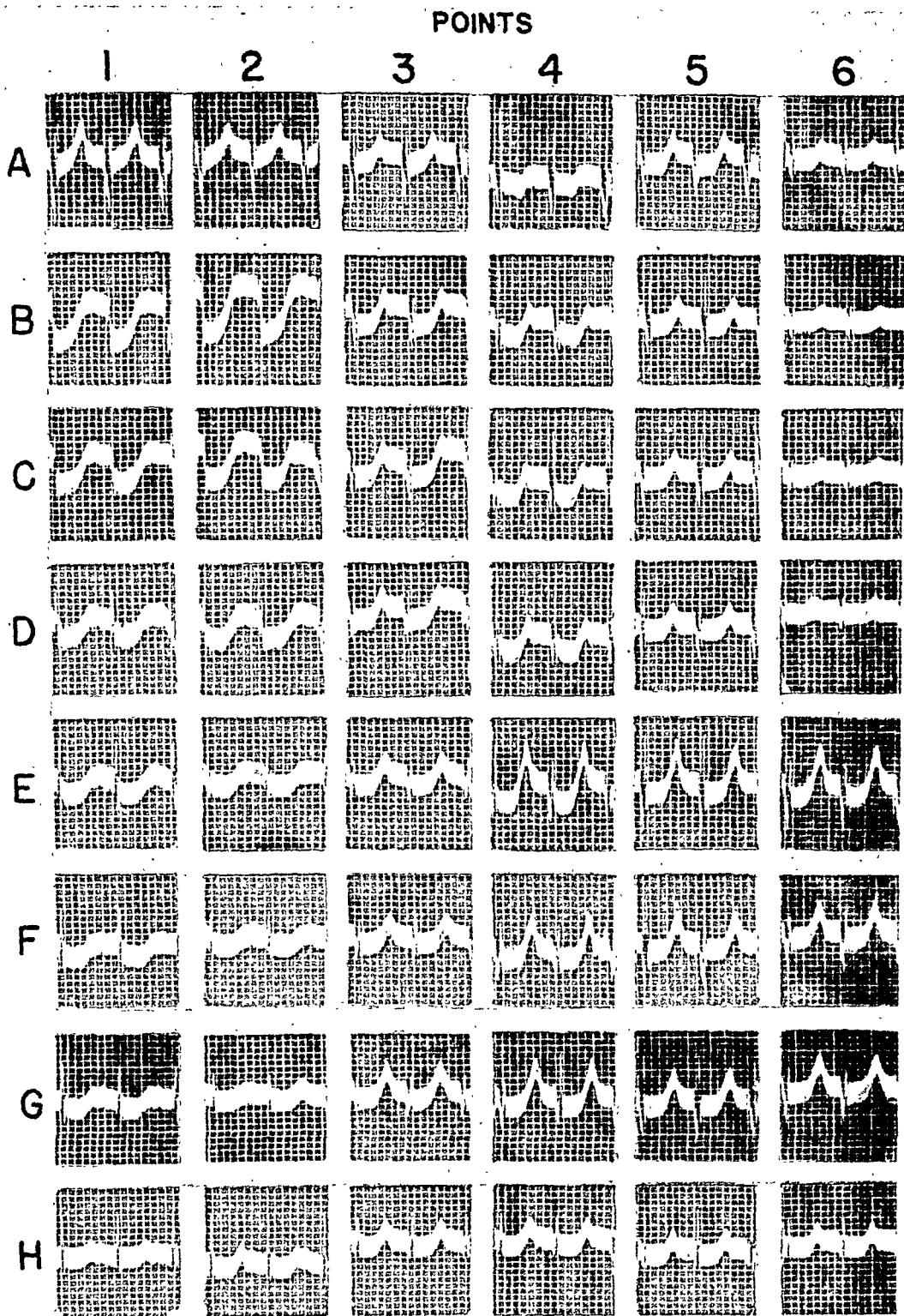
Fig. 7. Serial unipolar leads taken from 6 spots on the heart before and after intramyocardial injection of alcohol. Each vertical numbered column contains segments of records made from the same spot at various times. Spot 1 was approximately at the center of the alcohol-injected region; the remaining 5 spots were located in a straight line from spot 1 at approximately equal distances from each other spot, 5 being outside and 6 well out into normal tissue. Series A was taken before injection, and series B immediately afterwards. Series C, D, E, F, G, and H were taken 5, 15, 30, 45, 60, and 75 minutes after injection respectively. Discussed in text.

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The results obtained can be summarized as follows:

1. Changes in the contour of the QRS occurred first in the region in which alcohol was injected and later also in the other areas outside this region. This indicates that the pattern of activation of the heart in this region is altered, first by the original injury and later, by the subsequent effect of the initial injury on neighboring regions. The outstanding change
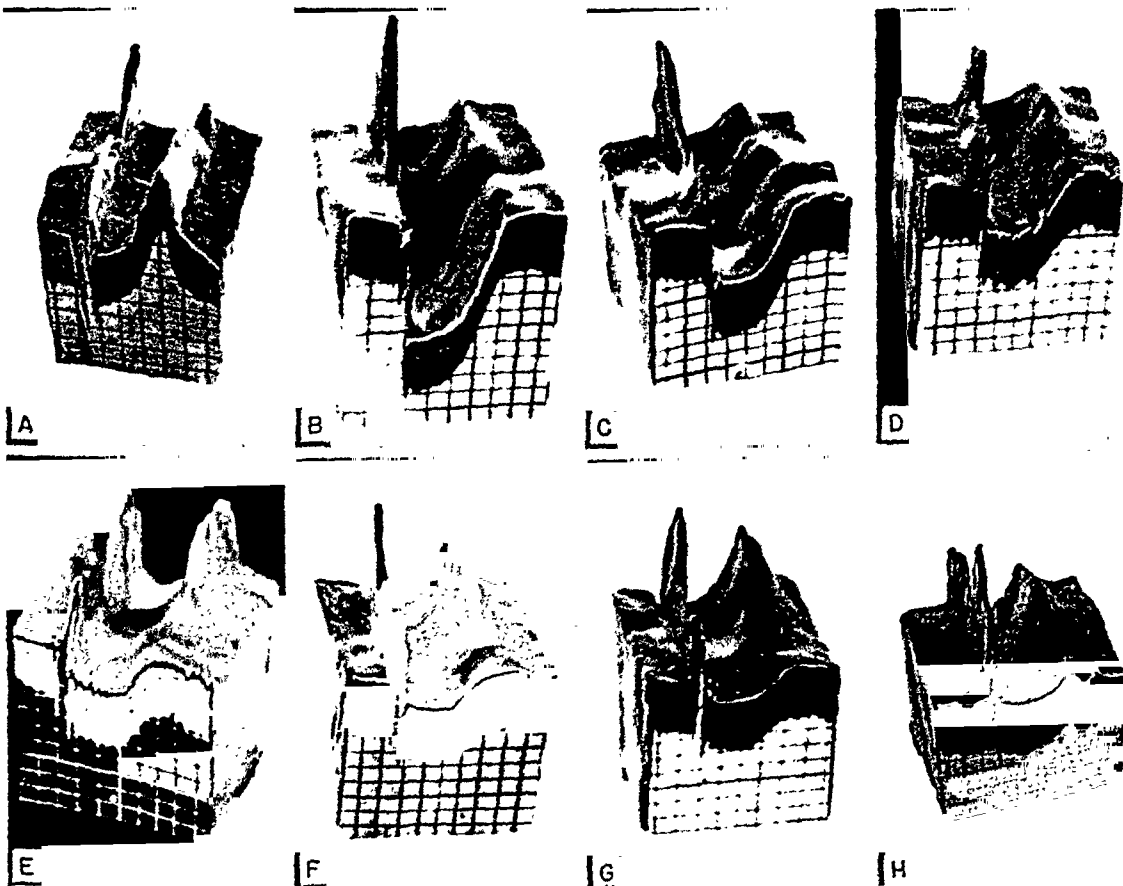
Fig. 8. Photographs of a series of three dimensional models, each model made from the correspondingly lettered series of figure 7. The horizontal axis represents time during the heart cycle; the vertical axis represents potential; and the axis at right angles to these two represents distance from the center of injury. Discussed in text.

was in the first inverted phase of the QRS and only slightly less was the effect on the subsequent positive phase. These experiments like those of Wilson et al. lend support to the view that alterations in the pattern of activation are probably responsible for the alterations in the QRS in indirect leads in animals and man which are seen to follow localized ventricular wall injury. In figure 8 these changes affect the contour of the precipitous QRS "canyon-mountain range."

2. Changes in the level of the S-T segment in the form of a depression at first confined to the region of the initial injury and its immediate environs' and being greatest in the center and less marked at the edge. Later, as the S-T depression in the center of injury decreases, this difference between the center and the edges of the injury lessened and the area over which the S-T depression was located extended outwardly. Eventually, this S-T depression disappeared or tended to do so. The mechanism for its production would be along the lines mentioned in the possibilities discussed in section I and illustrated in figure 4. It is most likely that possibility D is what actually occurs. Obviously, as restitution occurs with time and a new polarized surface is gradually established in the injured region, the variations in potential of this region between the resting and active periods of the ventricles will decrease. Because the injury is less at first at the margin than in the center of the alcohol injected area the potential variation during these two periods will be less in the former region. The slower restitution at the margins of the original area and the later extension of the injured area is attributed to the spread by diffusion and via the lymphatics of noxious material from the area originally injured by alcohol. This chain of events gave rise in figure 8 at first to a deep broad "valley" which midway back sloped steeply upward; later, this was replaced by a shallower more extensive "valley" and ultimately it disappeared.

3. The most striking finding, hitherto not fully described as far as we know, was the late development of a characteristic upright T wave (seg. E., fig. 7) confined *to the margin and the region surrounding* the original injury. This T wave tended to wane later, more rapidly in the outermost regions and, at the same time, it tended to appear in regions closer to the center of original injury (seg. G, fig. 7). Ultimately, it too tended to disappear (seg. H., fig. 7). The contour of this T wave is specific for the "coronary" T wave described in man,.namely, a peaked T wave, with symmetrical limbs, rounded shoulders and accompanied by an S-T segment which both deviates and bows in a direction opposite to the T wave. This T wave appeared in figure 8 as a broad "mountain range" in the back of the model which sloped downward from back to the front; later its tilt from front to back decreased, as did its maximum height and ultimately this T was replaced by the normal broken uneven T wave "range."

On a purely electrical basis, following the views for the S-T changes which Eyster, Meek et al. suggested and which are confirmed by our work, this T wave change must indicate the presence of two pairs of concentric rings at the time in the heart cycle when the T wave is recorded as shown in figure 6. The inner pair of rings would be located at the inner margin of the area where this T is recorded, with the positive charge on the inside and negative on the outside. The outer pair of rings would

be located at the outer margin of the area where this T is recorded and have the charges of each ring the reverse of the inner pair. This would limit this T configuration to the area enclosed between the inner and outer pair of rings.

This view can be correlated with the membrane theory. At the time the T wave is being written the process of restitution of the polarized state is occurring at the cell surfaces. It would, therefore, be logical to assume that the "coronary" T wave was an expression of an alteration in the pattern in which this restitution takes place, and it would readily be accounted for along the lines developed in section I and illustrated in figure 6 if it were assumed that the region where this T wave occurs was, because of injury, tardy in its restitution process while more or less normal in its responsiveness to activation. In other words, in the ring-like region surrounding the originally injured region, there is left a more or less normal depolarization process during activation, *but there is a retardation in the rate of repolarization so that this region temporarily lags behind the rest of the ventricles.* In figure 6 the membrane potentials are illustrated during this part of the heart cycle in a manner similar to figure 4.

It would appear from this hypothesis, that the "coronary" T wave in indirect leads following local myocardial injury in animals and in man could be explained as an electrical expression of the occurrence of regions in which the ability to respond to activation is not impaired to any extent but in which the injury has resulted in a retardation of the process of restitution of the polarized state. With the rest of the ventricle repolarizing at its normal rate, a new potential difference is thus established which will wax and wane during this phase of the heart cycle in the manner which the contour of the coronary T wave depicts.

Apparently, then, injury can cause not only 1, an alteration in the pattern in which the impulse spreads; 2, an injury current during rest, and 3, a lack of response of some regions, but in addition *a retardation of the recovery process in a region capable of responding more or less normally.* When the last occurs, the characteristic T wave appears. It is of note that this not only occurs later than the S-T deviation but originates in a different way and in a different region.

SUMMARY

The electrical changes produced by an area of injury on the dog's ventricle were studied in two types of experiments. In the first type, a very small injured area was produced by pressure, and by means of a unipolar lead from this spot, it was found that as the injury is produced, the spot becomes negative when the heart is at rest and positive during complete cardiac activation, measured with respect to the potential of normal uninjured cardiac muscle. These changes disappear on recovery from

the injury. This observation confirms the recent results of Eyster, Meek, et al. (6).

In the second type of experiment, a small area of injury was produced by intramyocardial injection of 95 per cent alcohol, and, by means of unipolar leads, the potential changes with time were followed in the injured area and at points on the ventricles at different distances from the center of injury. The results were:

1. Changes in the contour of the QRS complex, indicating an alteration in the pattern of impulse spread, occurred in the injured area and later also in other areas outside this region.

2. A depression of the S-T level occurred, which was maximum in the injured region and became smaller out toward the periphery. These changes tended to disappear with time.

3. A coronary type of upright T wave appeared some time after the injury was produced, and was confined to the margin of the injury and a narrow region surrounding it. This T wave tended to disappear with time.

An explanation of the results of both types of experiment is offered on the basis of the classical membrane theory.

1. The change in contour of the QRS produced by injury is ascribed to alteration in the pattern of impulse spread.

2. The T-Q elevation and S-T depression are attributed to the production by injury of a region which is partially depolarized at rest and irresponsive duration activation.

3. The late appearance of the large upright "coronary" T wave is ascribed to the production outside the original area of injury of a partially injured region which responds normally to activation, but which lags temporarily behind normal tissue in the process of repolarization.

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UTILIZATION OF THE KETONE BODIES IN NORMAL ANIMALS AND IN THOSE WITH KETOSIS

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The capacity of normal tissues to utilize ketone bodies was studied by Marriot (1) and later by Snapper and Grünbaum (2). The observations of these workers indicate that it is quite high. Chaikoff and Soskin (3) showed that diacetic acid injected into the depancreatized dog after hepatectomy was utilized by the other tissues of the body. Embden and his co-workers (4) found that the perfused liver added ketone bodies to the perfusing blood. Mirsky (5) showed that the ketosis caused by anterior pituitary extract needed the liver for its production. These observations together with more recent work (6, 7) points to the liver as the site of formation of ketone bodies in ketogenic states. In such conditions what is the rôle played by the other tissues of the body? Do they have a diminished capacity to utilize these substances and hence allow them to accumulate in the body or do they use these bodies at a normal rate, the ketosis resulting from a greatly increased production of them by the liver? The work reported in this paper is concerned with these questions.

Little accurate work has been carried out on the rate of ketone utilization of normal tissues or of the tissues of animals in a state of "ketosis." Chaikoff and Soskin (3) made measurements on their depancreatized dogs. They injected diacetic acid into these animals after hepatectomy and nephrectomy and followed the blood ketones thereafter. The injection carried the blood ketones to levels above 100 mgm. per cent, from which there was usually a rapid drop in the course of 2 or 3 hours to around 10–30 mgm. per cent, where there was a tendency to level off. These results would seem to suggest that the utilization rate was very high at the high levels but not of any great magnitude at levels found in ordinary conditions of ketosis. The recent work of Dye and Chidsey (8) supports this conclusion. Blixenkrone-Moller (9) measured the ketone body utilization of muscle by perfusing the hind quarters of cats and found that both normal and diabetic tissues burned ketone bodies at similar high rates. The concentration of the ketone bodies in the perfusing fluid was quite high.

The aim of our work was to measure the ketone body utilization in animals in ketosis, and in normals, with the blood ketone body concentration at the low levels ordinarily observed in "ketosis" and with a minimal change from the normal physiological state of the animal. This we carried out by the method of arterio-venous differences which has been previously described (10). The most important advantage of the method is that the tissues are not affected by the experimental procedures, as is the case in methods such as perfusion or tissue slice procedure, in which drastic changes may be made in the condition or environment of the tissue. Also the concentration of the ketone bodies in the blood, which may be a factor in determining the rate of utilization, is kept at the natural levels.

TABLE 1

| PREPARATION | ACETONE BODIES AS ACETONE | | A-V DIFF. AS ACETONE | A-V DIFF.* AS ACETONE BODIES | A-V DIFF. OXYGEN | PER CENT TOTAL METABOLISM AS ACETONE BODY OXIDATION |
	Arterial	Venous				
Diabetic rabbit.........	3.14	1.57	1.6	3.1		
Diabetic goat...........	3.15	2.40	0.8	1.5	6.40	23.4
Diabetic dog...........	6.75	6.25	0.5	1.0	9.43	10.6
Diabetic dog...........	11.5	9.5	2.0	4.0	2.00	200.0
Diabetic dog...........	13.6	11.9	1.7	3.2	10.75	29.9
Diabetic dog...........	24.1	22.0	2.1	4.3	5.50	78.0
Phloridzin rabbit.......	3.08	1.75	1.3	2.6	9.00	28.8
Phloridzin rabbit.......	3.20	2.20	1.0	1.9	3.50	54.3
Phloridzin dog..........	6.80	6.10	0.7	1.4	4.36	32.1
Phloridzin dog..........	7.90	6.80	1.1	2.1	5.99	35.0
Phloridzin dog..........	25.60	23.60	2.0	4.1	5.75	71.4
Phloridzin dog..........	29.75	27.45	2.3	4.7	5.75	81.6

* "Acetone bodies" are estimated by assuming that they are present as aceto-acetic acid (25 per cent) and β-hydroxybutyric acid (75 per cent).

The bloods were taken from the femoral vein and artery. The venous bloods were taken first. Nine cubic centimeter samples were collected, sufficient for both ketone body (11) and oxygen (Van Slyke manometric) determinations. Ketosis in the diabetic animals was brought about by withholding food and insulin. Phloridzin ketosis was produced by fasting and the daily injection of one gram of the drug in olive oil. The results are given in table 1.

In column 6 is given the per cent of total metabolism taken by acetone bodies assuming 1 mgm. of mixed ketone bodies requires 1 cc. oxygen for combustion. This column actually gives the fraction of oxygen consumed by ketone bodies. However the energy produced per cubic centimeter oxygen used in burning other foodstuffs in the tissues probably differs

little from that for the ketone bodies so that the figure given in column 6 would approximate the percentage of total metabolism produced by burning of ketone bodies. In one case (diabetic dog 3) the figure is impossibly high. This is obviously due to the very low oxygen A-V difference. This could be caused by a temporary rapid blood flow which would lower the oxygen A-V difference, but would hardly affect that for the ketone bodies since their concentration in the tissues would be little affected by sudden changes in blood flow and it is this concentration which is in equilibrium with the venous blood. The average for all values in column 6 excluding that of 200 per cent is 44.5 per cent. It is to be noted that these values tend to be higher when the blood ketone concentrations are higher, which supports previous work (8).

Utilization in normals. The utilization of normal animals was carried out by determining the rate at which sodium dl-β-hydroxybutyrate could

TABLE 2

ANIMAL	WEIGHT	TIME OF INJECTION	AMOUNT INJECTED	AMOUNT EXCRETED	KETONE BODIES, BLOOD MGM. PER CENT		UTILIZA-TION, PER KILO MIN.*
					Start	End	
	kgm.	*minutes*	*mgm.*	*mgm.*			*mgm.*
Rabbit 1......	2.66	17	74	0.7	3.9	3.9	1.62
Rabbit 2......	2.78	25	120	12.1	10.5	11.7	1.31
Rabbit 3......	2.08	23	101		7.6	6.7	2.30
Dog 1A........	13.3	16	256	1.3	5.3	4.85	1.39
Dog 1B........	13.3	13.5	205		6.0	5.3	1.80

* In calculating these figures a correction for the change in concentration in the tissues was made. It was assumed that 70 per cent of the body weight was water diluting the ketone bodies in the case of the dog, and 50 per cent for the rabbit.

be injected intravenously into them without causing a change in the blood concentration. In such a steady state the rate of injection minus the kidney excretion represents the rate of utilization by the tissues. Rates satisfying such a requirement were first established by trial and error in preliminary experiments. Thereafter our procedure was as follows: we injected the solution of sodium dl-β-hydroxybutyrate (0.4 per cent in saline) rapidly at first in order to bring the blood ketone level into the region of that of our "ketosis" animals and then injected at the steady rate we wished to study, taking blood samples for ketone determination at the beginning and end of the period. The results are given in table 2.

We may obtain an approximate idea of the utilization rates of our ketosis animals if we assume that the venous blood values are the same as those for the mixed venous blood. The utilization rates would then be the product of the A-V differences by the minute volume of the heart.

The values for our ketosis animals with low blood ketone levels are given in table 3. Cardiac outputs for the dog are from Marshall (12), for the goat from Barcroft et al. (13) and for the rabbit from Dock and Harrison (14). The utilization rates so estimated are if anything greater than in the controls.

The oxygen and ketone body arterio-venous differences were determined in some of the control animals and are given in table 4.

These differences on the average are smaller than those obtained on "ketosis" animals. This is probably due to the fact that the levels are rather low and also that but one of the ketone bodies was injected, whereas

TABLE 3

Ketone body utilization rates of "ketosis" animals which had blood ketone body levels in the same range as that of the control animals

ANIMAL	UTILIZATION, PER KILO MIN.
	mgm.
Diabetic goat	2.0
Diabetic dog	1.45
Phloridzin dog	2.03
Phloridzin dog	2.9
Diabetic rabbit	3.7
Phloridzin rabbit	3.1
Phloridzin rabbit	2.3

TABLE 4

ANIMAL	KETONE BODY DIFFERENCES	OXYGEN DIFFERENCE
	mgm. per cent	*cc. per cent*
Rabbit 2	1.0	2.2
Dog 1A	0.4	1.5
Dog 1B	1.2	5.5

the liver of the ketogenic animal adds both acetoacetic and β-hydroxy-butyric acids to the blood. The liver is capable of changing β-hydroxy-butyric acid to acetoacetic to some extent (15). There is nothing in these results that would indicate that the tissues of "ketosis" animals are any less able to utilize ketone bodies than normal animals. It seems hardly necessary to compare the capacities of the two types of animal at higher levels: the "ketosis" animals there obtain such a high percentage of their energy from ketone body burning that the normal could not possibly be much higher.

The rôle of ketone bodies in normal metabolism. A question arises when one considers that such a large fraction of total fat metabolism is carried

out through the intermediary steps of ketone bodies; is this a necessary
preparation of the fats for the ready utilization of them by tissues like
the muscles? It does not seem to be absolutely so since in fasting animals
after hepatectomy (which stops all ketone body formation) there is good
evidence of utilization of fat (16). The hepatectomized animal remains
fairly quiet and has practically a basal metabolic rate. Would he be
capable of carrying on a high metabolic rate as a result of exercise without
the aid of this intermediary change of the fats if they were the predominant
source of fuel? The utilization of ketone bodies by skeletal muscles is
increased by having them work (9) although little change in ketone excre-
tion or ketonemia level is brought about in the body as a whole as a result
of exercise. It has been shown by one of us (17) that a subject on a con-
stant ketogenic diet may tend to excrete somewhat more ketone bodies
in the urine on days of inactivity than on days of large expenditures of
energy. The differences are not large, however.[1]

TABLE 5

Blood ketone bodies in milligrams per cent

	1 DAY	2 DAY	3 DAY
8 a.m.	26.6	26.4	35.9
12 noon	10.3	9.5	8.8
3 p.m.		16.3	
Urine 8 a.m. to noon in mgm.	472	278	538

We made observations on a subject (75 kgm. wt.) on a constant ketogenic
diet. On three successive days, with no breakfast, bloods were taken for
ketone determinations at 8 a.m. and noon, and the urine was collected for
the period. Between the time of the blood samples of the first morning
the subject stayed in bed, on the second morning he engaged in heavy
exercise and on the third he engaged in light exercise. On the second
day after the noon blood was taken the subject continued without food
but stopped the exercise, and at 3 p.m. a third blood was taken. The
results are given in table 5.

The differences in urine excretion are relatively unimportant in com-
parison with the large amounts of ketone bodies which must have dis-
appeared from the body in order to lower the blood level of them to such
a degree. The heavy exercise of the second day did not cause any greater
drop in blood ketone level than occurred in the control days. These
results are not incompatible with the findings of Blixenkrone-Moller (9)
which show that exercise increased the utilization rate of ketone bodies.

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[1] Since this was written an article by Barker (J. Physiol., Vol. 97, No. 3) has
appeared which reports similar findings.

These seemingly contradictory statements can be reconciled if we suppose that in "ketosis" states, the liver during exercise increases its production of ketone bodies commensurate with the increased utilization of them by the muscles and so leaves the balance between production and consumption the same as during rest.   The blood ketone rise at 3 p.m. on the 2nd day of our series gives some support to this theory.   We may best presume then that our present knowledge indicates that the ketone body intermediary transformation is not necessary for the combustion of fats but may be a supplementary mechanism operating particularly when the body must expend large amounts of energy with fat as the fuel.

DISCUSSION.   Our findings give no support to the concept that one molecule of fatty acid can give rise to but one molecule of ketone.   The results indicate that from 30 to 80 per cent of the energy requirements of the tissues in ketogenic states may be supplied by combustion of ketone bodies.   In the conditions produced, around 80 per cent of the ultimate source of energy was fat.   This would be made up predominantly of long chain fatty acids, and one cannot account for such a large ketone body production and utilization if each molecule of fatty acid gave rise to but one molecule of ketone body.   Butts (18), Deuel (19) and their co-workers using rat feeding experiments have shown that the urinary acetone body production is greater than can be accounted for by a one to one ratio. Blixenkrone-Moller (20) from observations on perfused diabetic cat livers concludes that 4 molecules of ketones may be formed per molecule of fatty acid.   The recent work of Stadie, Laff and Lukens (21) with liver slices supports such a view.   We have therefore abundant support in the literature for the higher than one to one ratio between fatty acid and ketone which is necessary to explain our results.

SUMMARY

We have determined the ketone body utilization of animals in a state of ketosis caused by pancreatic diabetes or phloridzin.   The method used was that of simultaneous ketone body and oxygen arterio-venous differences.   The average fraction of the oxygen difference utilized by burning ketone bodies was 44 per cent.

Normal animals injected with β-hydroxybutyric acid show no greater utilization of ketone bodies than animals with ketosis.

We have presented the view that the production of ketone bodies by the liver and utilization of them by the other tissues is an important, though not necessarily inevitable, route for the catabolism of fatty acids. When the organism is in a state of ketosis, increases in metabolic rate (as in exercise) probably increase the rates of production and utilization of these substances.

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THE CAPACITY FOR VIGOROUS MUSCULAR ACTIVITY OF NORMAL RATS AND OF RATS AFTER REMOVAL OF THE ADRENAL MEDULLA

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The bulk of the experimental evidence on the function of the adrenal medulla seems to indicate that this portion of the gland plays no significant rôle in bodily economy during periods of rest, but that in conditions of emotion, asphyxia, exposure to cold and violent muscular exercise epinephrine is liberated reflexly. Functional changes following the injection of epinephrine and those following stimulation of the sympathetic nervous system are similar and Cannon has pointed out that these functional responses are useful in preparing the animal for activity in conditions of stress. The effects observed when epinephrine is injected include acceleration of the heart and augmentation of the heart beat, constriction of the blood vessels of the skin and splanchnic regions, increased blood pressure, relaxation of the bronchioles, deepened respiration, glycogenolysis in the liver, hyperglycemia, and release of erythrocytes from the spleen. All of these are valuable physiologic reinforcements in situations in which intense muscular activity is demanded. The functional importance of the adreno-sympathetic mechanism in mediating these important physiologic responses during emergencies is well established. Studies on animals in which the adrenal medulla has been destroyed by a method which does not limit the functional activity of the adrenal cortex, and in which the rest of the sympathetic system has been left intact, however, have given little convincing evidence that animals following destruction of the adrenal medulla are less capable of survival than a normal animal in a condition of stress which involves vigorous muscular activity.

Ingle, Hales and Haslerud found that destruction of the adrenal medulla did not limit the capacity of the rat to continue the work of the stimulated gastrocnemius muscle. In a recent study, one of us (D. J. I.) (4) observed that during the first few hours of stimulation the height of muscular contraction is maintained much better by normal rats than by rats from

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[1] Now residing in Philadelphia.

which the adrenal medulla has been removed. However, after a few hours
the amounts of work performed became similar and the total amount of
work performed before the loss of muscular responsiveness by rats after
destruction of the adrenal medulla was just as great as that of normal rats.
Ingle and Harris observed that the voluntary activity of rats after destruc-
tion of the adrenal medulla was normal. Richter confirmed this observa-
tion. Campos, Cannon, Lundin and Walker found that inactivation of
the adrenal medulla of the dog did not limit its capacity for prolonged
work on the treadmill.

In the present study we have compared the time taken for normal
rats and for rats after removal of the adrenal medulla to swim until ex-
hausted. The "emergency" type of situation described by Cannon is
more nearly approximated than in previous experiments.

METHODS. The adrenal glands of male rats of the Wistar strain were
enucleated by technic described by Evans. The operations were per-
formed when the animals weighed from 45 to 85 grams. Similar rats had
incisions only. All operations were performed with the animals under
ether anesthesia and sterile technic was used.

The tank used for swimming was made of galvanized iron, 22 inches
(55.5 cm.) deep and 17 inches (42.5 cm.) in diameter. It was filled to a
uniform depth for each series of tests with water held at a constant tem-
perature of 30°C. All the tests were made at the same time of day. The
animals were fasted for twenty-four hours before the tests. Since rats
are capable of swimming continuously for several hours before exhaustion
the time was shortened by tying a weight close to the proximal end of
the tail. With a weight of 10 grams the average time required for exhaus-
tion was from ten to fifteen minutes; with a weight of 20 grams, two to
three minutes. The experimenter (R. E. H.) who made the observations
on swimming time was not aware of the identity of the animals at the
time the tests were made.

EXPERIMENTS AND RESULTS. A preliminary experiment was performed
in which a group of rats which had been used in previous experiments
swam with 20 gram weights attached to their tails. These animals were
heterogeneous in body weight. Twenty-five rats in which the adrenal
medulla had been destroyed were compared to twenty-three animals
which had been subjected to incision only. There was no significant
difference in the average time required for exhaustion in the two groups.

In experiment 1, the animals were forced to swim when they reached
a body weight of 180 grams with a weight of 20 grams. There was no
significant difference in the average time required for the rats in the two
groups to swim to the point of exhaustion. In experiment 2, the animals
were weighted with 10 grams, thus lengthening the time required for ex-
haustion. The rats without adrenal medullas were superior to their con-

trols, this time to a point approaching the usual criterion for statistical significance. Since there is no obvious reason for the differences in favor of the animals without adrenal medullas, we are inclined to attribute the difference found to the vagaries of small sample in an undefined but probably homogeneous population.

Twenty of these animals were retested later, not less than two weeks after the first test. The coefficient of correlation between the scores on the test and retest was $+0.90 \pm 0.03$. The results of experiments 1 and 2 are presented in table 1.

COMMENT. It is reasonable to conclude that under these experimental conditions the absence of the adrenal medulla does not decrease the capacity of the rat to respond in a normal manner to this type of "emergency" situation. To generalize beyond these experimental conditions to the rôle of the adrenal medulla in all conditions of stress is not justifiable. It was not established by these experiments that there is a reflex discharge

TABLE 1

Time required to swim to point of exhaustion: rats with and without adrenal medullas

EXPERI-MENT	ADRENAL MEDULLA	NUMBER OF RATS	WEIGHT ATTACHED	AVERAGE TIME	DIF-FERENCE	FISHER'S t*
			grams	seconds	seconds	
1	Removed	28	20	137.25		
	Not removed	28	20	133.86	3.39	0.404
2	Removed	27	10	719.32		
	Not removed	23	10	597.71	121.61	2.04

* Fisher, R. A. Statistical methods for research workers. Edinburgh, Oliver & Boyd, 1930, p. 108.

of epinephrine from the adrenal medulla during vigorous swimming. Unfortunately, there is no satisfactory test for the presence of epinephrine in the blood of the rat. Moreover, there are other possible sources of epinephrine or epinephrine-like substances which are not eliminated by the destruction of the adrenal medulla.

SUMMARY

Normal rats and rats after removal of the adrenal medulla were compared in respect to the times required to swim to exhaustion when they were handicapped by weighting. The performances of rats without the adrenal medullas were as good under these experimental conditions as those of normal rats.

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THE INTERACTION OF CENTRAL AND PERIPHERAL CHEMICAL CONTROL OF BREATHING[1]

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Before the chemoceptive function of the carotid and aortic bodies was known, the chemical control of breathing seemed a relatively simple problem. Lack of O_2 and excess of CO_2 were regarded as normal respiratory stimuli operating solely at the respiratory center, and the changes in pulmonary ventilation of such chemical origin appeared to harmonize with changes in acidity of the respiratory center (Gesell, 1925, 1929). But when reflexogenic chemical control (Heymans, Bouckaert and Dautrebande, 1930) as well as centrogenic control of breathing was established new problems developed. Not only was it desirable to know the relative parts played by centrogenic and reflexogenic control, and the response of the center and of the chemoceptor to O_2 lack and CO_2 excess, but it was of equal interest to determine the interaction of the central and peripheral mechanisms. Our present experiments bear on these fundamental issues and we believe offer a simple reconcilation of facts with the acid mechanism of control.

METHOD. Our method was relatively simple. It consisted essentially of temporary bilateral blocking and deblocking of Hering's nerve during normal and modified breathing. The vagus nerves were sectioned to permanently eliminate those chemoceptor signals arising in the aortic bodies and to abolish interfering pressure reflexes arising in the aortic arch. Hering nerve block, therefore, prevented all known remaining chemoceptive signals from reaching the center and thus revealed breathing of purely centrogenic origin. Deblocking returned the reflexogenic component.

The cold blocks were made of copper, shaped to fit neatly into the region of the nerve after removal of the larynx. They were chilled and warmed with rapidly circulating alcohol. Temperature changes between 37°C and −3°C required 30 seconds. The moment of blocking and deblocking was signaled when the temperature reached 0°C and 30°C respectively.

[2] A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.

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In preliminary experiments it was found that temporary Hering nerve block during eupnea might produce one of three effects,—increased breathing, decreased breathing, or no observable change. Such variability of results, noted by others as well (see Stella, 1935), is readily explained by the simultaneous elimination of two sets of signals ascending Hering's nerve: *a*, excitatory signals coming from actively discharging chemoceptors of the carotid bodies, and *b*, inhibitory signals coming from stretched endings of the carotid sinuses. Should the inhibitory action of the carotid sinus be greater than the excitatory action of the carotid body an increased volume of breathing would be expected to occur during nerve block. On the other hand, should the excitatory action of the carotid body be greater than the inhibitory action of the carotid sinus decreased breathing would occur. Should both actions be equal there would be no change in breathing at all.

The correctness of these assumptions was confirmed by further orientation experiments in which blood pressure changes were automatically compensated at relatively low pressure levels or in which the carotid sinuses were collapsed. Under these circumstances block never produced an increase of breathing. There was either a diminution or no effect at all. Each sinus was, therefore, routinely collapsed.[3]

Our observations were made exclusively on dogs (anesthetized with morphine (3.5 mgm/kilo) and chloralose (100 mgm/kilo)) under the following conditions:

1. During hypocapnia produced by excessive artificial ventilation with a room air mixture.

2. During acute hypercapnia produced by the administration of 10 and 15 per cent CO_2 mixtures in 65 per cent O_2.

3. During progressive hypercapnia produced by rebreathing a small volume of a high O_2 mixture without reabsorbing the exhaled CO_2.

4. During acute O_2 lack of graded intensities produced by breathing varying O_2 mixtures in N_2 (40 to 6 per cent O_2 in N_2).

Centrogenic and reflexogenic breathing in the eupneic range of chemical

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[3] The sinuses were relieved of their normal distention by tying one ligature around the common carotid artery approximately an inch below the sinus, a second around the internal carotid just distal to the sinus and a third around the external carotid above and as close to the sinus as possible (Gollwitzer-Meier, 1934) and then puncturing the common carotid peripheral to ligature 1. As Winder (1933) and Winder, Bernthal and Weeks (1938) point out, most effective anastomoses act to preserve a uniform flow of blood through the carotid body on occluding the common carotid artery. A concomitant rise in systemic pressure from elimination of the sino depressor reflex acting through the circle of Willis would tend to maintain a uniform head of pressure in the occipital artery supplying the carotid body. In that event our results would give a fairly reliable indication of the relation rôle of the chemoceptors during eupnea.

stimulation. The capacity of the center and chemoceptor to respond to the major respiratory stimuli (i.e., O_2 lack or CO_2 excess) is now accepted as fact. (For references see Heymans and Bouckaert, 1939; Gesell, 1939; and Schmidt and Comroe, 1940.) The similarity of the respiratory tracing during CO_2 administration, before and after chemoceptive denervation, leaves no doubt of the capacity of the center to respond to chemical changes occurring within itself. The smallness or the absence of response to O_2 deficiency after chemoceptive denervation shows the effectiveness of reflexogenic breathing. Despite the general agreement on this point considerable discussion still remains regarding the relative effectiveness of CO_2 excess and O_2 deficiency at the center and chemoceptor respectively. Heymans and his associates (1939) insist on the predominating rôle of the carotid body, for CO_2 as well as O_2 regulation. This view was supported by the intense hyperpnea which they and others produced by a localized hypercapnia in the vascularly isolated carotid body. They pointed to the significant observation that this hyperpnea persisted despite an undoubted overventilation and an hypocapnic condition of the respiratory center.

Comroe and Schmidt (1938), Schmidt and Comroe (1940) and Schmidt, Dumke and Dripps (1939), however, arrive at opposite results and conclude 1, that the vascularly isolated carotid body exhibits a low reactivity to changes of arterial carbon dioxide and oxygen; 2, that denervation of the carotid and aortic bodies produces no uniform effect upon alveolar carbon dioxide and, therefore, has no important effect upon eupneic breathing, and 3, that denervation neither retards nor diminishes the respiratory response to carbon dioxide. In their opinion "Carotid body reflexes constitute an accessory mechanism, brought into action by emergencies such as foreign chemicals, anoxemia, and unusually great increases in the CO_2 tension of the blood, rather than an essential part of the normal respiratory regulating system; the control of breathing under ordinary conditions is accomplished entirely by the direct effects of chemical stimuli (mainly CO_2) upon the cells of the center." The denervation experiments of von Euler and Liljestrand (1936) differ in turn from those of Schmidt and Comroe. They found an increased alveolar CO_2 pressure after denervation during eupnea and interpreted this change as a sign of diminished breathing. Bernthal and Weeks (1939) found that breathing and vasomotor activity were reduced when the carotid bodies were cooled. Bogue and Stella (1935), Samaan and Stella (1935) and von Euler, Liljestrand and Zotterman (1939) found low CO_2 thresholds for activation of the carotid body and Bernthal (1938) found a reaction to small changes in carbon dioxide pressures. These results must be interpreted to mean that the center and the chemoceptors participate jointly in the control of eupneic breathing and that a higher intensity of chemical stimulation

is required to drive the respiratory machine when the chemoceptors are out of function. Our own orientation experiments already cited indicate the same for at least a portion of the animals.

The central response to oxygen deficiency is either missing or decidedly diminished when tested under anesthesia (see reviews of Heymans and Bouckaert, 1939; Gesell, 1939; Schmidt and Comroe, 1940) and, therefore, must be of little practical value to the animal. Reflexogenic breathing is without doubt the important component under such conditions. Only in the absence of anesthesia is central hyperpnea said to approach hyperpnea in the intact animal, (Dautrebande, 1939) a finding denied by Bouckaert, Heymans and Samaan (1938). While Schmidt and Comroe (1938) find a relatively high threshold for anoxemia in the carotid body preparations, Bernthal (1938) and von Euler, Liljestrand and Zotterman (1939) find the threshold within the eupneic range of oxygen pressure.

Our methods under conditions 1, (hypocapnia from overventilation) yield further information on the relative effectiveness of centrogenic and reflexogenic breathing during eupnea with a slightly different procedure. Dogs were connected with rebreathing tanks containing room air. Respiratory stimulation was then diminished by artificial overventilation of the lungs, sufficient to reduce or stop natural breathing after artificial ventilation was ended. As soon as standard conditions yielding a dependably uniform series of apneas or subnormal respiration had been established, Hering's nerves were blocked at the end of every second period of artificial ventilation. They were deblocked after natural breathing had returned. In the first of the two experiments used to illustrate our results, the respiratory tracing is seen to begin in eupnea and was presently followed by two minutes of artificial ventilation (see upper record). As indicated by the horizontal bar, nerve block began about one minute before the end of artificial ventilation and deblocking occurred shortly after the end of apnea. Whenever the chemoceptor signals were blocked in this experiment nearly one minute was required to rebuild a stimulus strong enough to interrupt the apnea produced by overventilation. But when the centrogenic and reflexogenic components were allowed to complement each other, breathing started immediately after cessation of ventilation. In the second experiment, in which overventilation was more effective (see records 2 and 3 of fig. 1) the duration of apnea was increased threefold whenever Hering's nerves were blocked. This was interpreted to mean that the threshold stimulus required to reinitiate breathing after the production of apnea is lower for the intact respiratory mechanism than for the center alone, working without the aid of the chemoceptor signals.

It seems most significant that the rôle of the chemoceptors should be so strikingly revealed in the duration of apnea when their influence upon the depth of eupneic breathing is disproportionately less (see the effects of

blocking and deblocking in the lower record). For that reason the shortening of apnea might readily be interpreted as a different phenomenon from that of the tonic stimulation of the center. But if alveolar oxygen pressures fell more precipitously than carbon dioxide pressures rose during apnea it is probable that breathing was reinitiated at a moment when both oxygen and carbon dioxide pressures were below eupneic levels, as happened in Haldane's experiments on man (1922). Oxygen lack, thereby, becomes the logical initiator of breathing in our experiments on
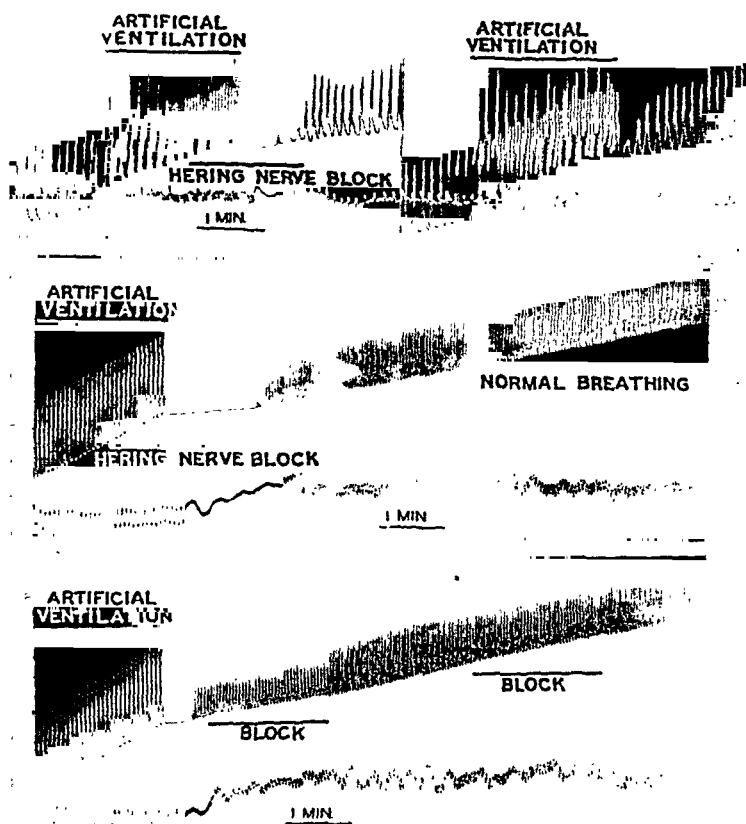
Fig. 1. Prolongation of apnea by withdrawal of reflexogenic support

the dog as well as in those on man. But in reaching this conclusion it is essential to remember that the effects of oxygen and carbon dioxide cannot possibly be separated if cH is the common stimulus to both. Lack of oxygen which leads to lactic acid formation must of necessity decrease the buffer base and increase the effectiveness of the prevailing carbon dioxide pressures. Theory, therefore, demands that the subeupneic carbon dioxide pressures at the moment of reinitiation of breathing contribute towards the stimulation of oxygen lack. Haldane's belief that lack of oxygen in some way increases the excitability of the center to carbon

dioxide agrees with this conception. This reasoning implies great responsibility of the chemoceptors as tonic controllers of oxygen pressures within the normal range of physiological stimulation.

Hypercapnia. The effects of high concentrations of carbon dioxide before and after deafferentation of the carotid and aortic bodies have already been described. The fact that only one comparison is possible upon a single animal makes a quantitative analysis of the relative importance of centrogenic and reflexogenic breathing difficult. This disadvantage is overcome in our experiments. Dogs were connected, as usual, with rebreathing tanks containing high carbon dioxide mixtures (10 to 15 per cent CO_2 in 65 per cent O_2 in N_2) and after hyperpnea had been well established Hering's nerves were alternately blocked and deblocked. The nerves remained unmolested in blocking position during comparative observations which eliminated the possibility of mechanical disturbance. Though the continuity of recording permitted a detection of the smallest changes in pulmonary ventilation, at no time did we notice a change in either the depth or frequency of the hyperpnea on nerve block. Subsequent tests with low O_2 or cyanide showed that the nerves must have been in good condition. Whatever the interpretation of our results may be, we see for the moment that the findings are not in complete accord with a predominant rôle ascribed to the chemoceptors in CO_2 control by Heymans. On the other hand if 10 or 15 per cent CO_2 in the inspired air can be regarded as an "emergency" they are in no better agreement with the position of Schmidt and Comroe whose schematic representation of centrogenic and reflexogenic breathing shows a powerful peripheral stimulation at high CO_2 pressures (1940).

This lack of reflexogenic breathing at high CO_2 pressures is in accord with the general evidence from many groups of experiments mentioned above on hypercapnia, before and after denervation. It is puzzling in face of the common findings that local carotid body activity actually does increase with increasing CO_2 pressures as is so very clearly indicated by the increased breathing produced by *localized* hypercapnia and by the linear relation of frequency of chemoceptive impulses to CO_2 pressures, ranging up to 14 per cent in the inspired air (von Euler, Liljestrand and Zotterman, 1939). Could it be that a generalized hypercapnia abolished in some way the central action of the signals which CO_2 set up in the periphery? And if this were true, at what pressures does the reflexogenic component fall out? These questions were studied by administering a 65 per cent O_2 mixture in N_2 with the aid of a rebreathing tank and allowing a rapid accumulation of the expired CO_2 in a limited volume of gas. Hering's nerves were blocked and deblocked and gaseous samples extracted from the tanks at appropriate intervals. Reference to figure 2 reveals the type of results obtained. It will be seen at once that breathing was

reduced by nerve block at the lower CO_2 pressures (expressed in per cent of CO_2 in the inspired O_2 mixtures) but not at the higher pressures and that the effect of blocking was no longer noticeable when the CO_2 in the inspired air had increased to approximately 5 to 6 per cent. We have, therefore, arrived at a rather paradoxical conclusion regarding the rôle of the carotid bodies. As physiological controllers of CO_2 pressures they are least effective when subjected to powerful stimulation and most effective when subjected to weak stimulation.

An explanation of the vanishing reflexogenic component was suggested by the earlier experiments of Gesell and Moyer (1935) in which hypercapnia was found to reduce or abolish respiratory reflexes, such as retardation and acceleration of breathing produced by central stimulation of the vagus and saphenous nerves respectively. Does carbon dioxide also abolish the central action of the very signals which it sets up in the carotid bodies? We believe it does.
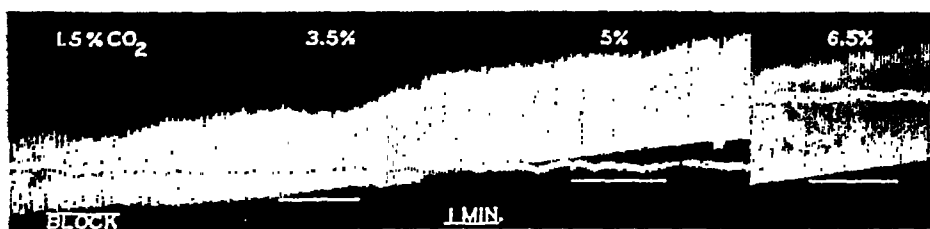
Fig. 2. Effects of blocking chemoceptor signals on pulmonary ventilation during progressively increasing hypercapnia.

Though our findings are incompatible with a predominant rôle ascribed to the carotid bodies by Heymans when carbon dioxide pressures are high they agree better with his views when the pressures are low. They fail, however, to harmonize with the views of Schmidt and Comroe at either high or low carbon dioxide pressures. According to their statement and curves (1940) they "actually found" an increasing reflexogenic component which at high carbon dioxide pressures was greater than the centrogenic component. So far as we are aware no confirmation of such results exists in the literature and our results indicate a progressively decreasing reflexogenic component replaced by an increasing centrogenic component as carbon dioxide pressure increases. The curves of Schmidt and Comroe were probably compiled from data taken during localized carotid body hypercapnia without consideration of the functioning of the respiratory mechanism as a whole.

Oxygen lack. Typical effects of O_2 lack on centrogenic and reflexogenic breathing are shown in the nerve block tests of figure 3 in which a dog successively breathed five mixtures of oxygen in nitrogen (40.0 per cent, 19.7 per cent, 16.6 per cent, 12.5 per cent and 8.6 per cent). The tests

began with a high oxygen mixture and showed the diminished breathing which so often occurred during Hering nerve block when the arterial blood was supposedly saturated with oxygen.   Granting an absence of ischemia in the carotid bodies, the centrogenic breathing which remained and the reflexogenic breathing which was removed by block were probably the result of the stimulating action of CO_2.   The effects of oxygen
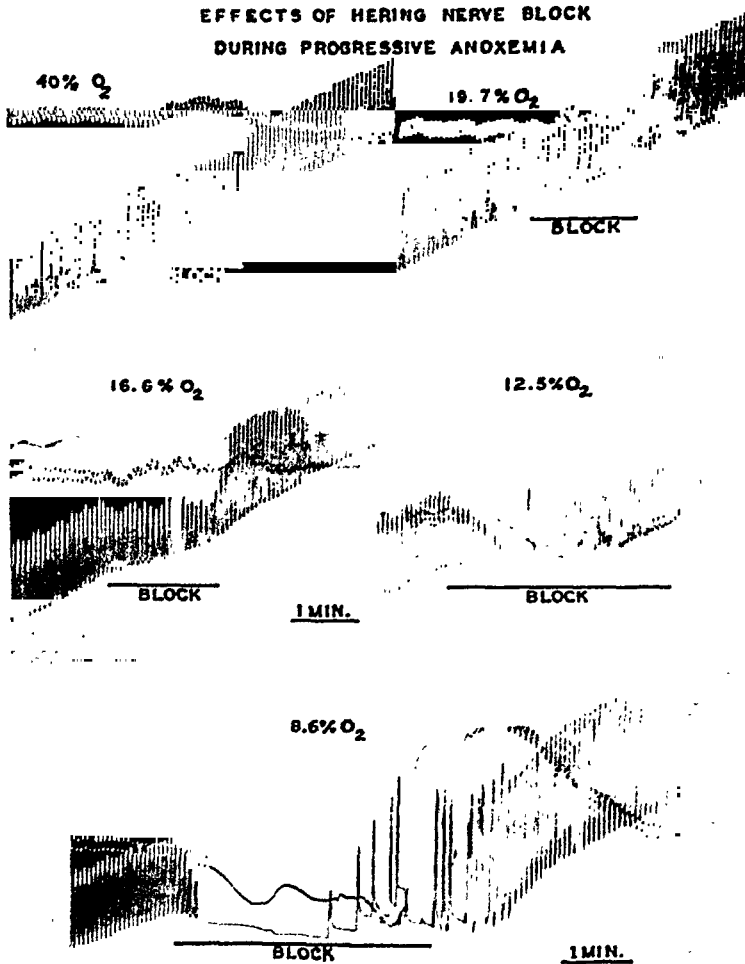
Fig. 3. Effects of withdrawing reflexogenic support during progressively increasing hypoöxia.

lack were as readily demonstrated when the oxygen of the inspired air was lowered.   It will be seen that when the dog was simply switched from the 40 per cent O_2 mixture to the 19.7 per cent O_2 mixture, breathing was augmented.   This in itself is indicative of a sensitive response of the respiratory mechanism to a slight reduction of arterial oxygen pressure for hyperventilation of this kind is always associated with a marked drop in carbon dioxide pressures.   That the respiratory stimulation actually

occurred in the chemoceptor is seen in the large reduction of breathing which occurred when the chemoceptor signals were blocked (compare the results of the first and second blocks). Since the centrogenic breathing in the second observation was less than in the first, reflexogenic breathing must have been increased by the oxygen deficiency. The hyperpnea, which prevailed before nerve block, occurred despite a diminished central support.

This diminished central support became more marked as rebreathing continued and oxygen want increased. In the experiment under consideration it reached its limits in the prolonged apnea when the oxygen in the inspired air stood at 8.6 per cent. Such diminishing central support is a most significant phenomenon in relation to the chemical mechanism of respiratory control. It is conceivably due to two causes. One is the ultimate paralyzing action of oxygen lack. The other is the alkalinizing effects related to increased elimination of CO_2 from increased ventilation of the lungs and blood, increased volume flow of blood, and increased CO_2 carrying capacity and pH of the blood. As the alkalinizing influence of oxygen deficiency increases, either from an increasing ventilation or from a long continuance of hyperpnea, centrogenic stimulation would decrease in proportion. Therefore, successive Hering nerve blocks would be expected to reveal a decreasing magnitude of centrogenic breathing. This view was expressed some years ago (Gesell, Krueger, Nicholson, Brassfield and Pelecovich, 1932) on the basis of direct measurement of the amount of CO_2 eliminated and of the increase of the respiratory quotient during anoxemia.

An acid interpretation of the diminishing centrogenic breathing during oxygen deficiency is of course tenable only on the assumption that the so called central "paralysis" is not an important factor. The sudden increase of pulmonary ventilation occurring at the moment of deblocking of Hering's nerves indicated a fitness of the centers for they responded immediately to the burst of signals released from the carotid bodies. The sudden and pronounced acceleration of breathing produced by deblocking of the vagus nerves (not illustrated) showed a similar fitness of the centers to react to proprioceptive signals. We are, therefore, inclined to believe that the apneas noted at low oxygen pressures were not paralytic, that the hyperpneas were reflexogenic and occurred despite a condition of central hypocapnic apnea. In this connection it is well to recall that hyperpnea and central acapnia are not incompatible. As Gesell and Moyer (1935) showed, a center made apneic by the injection of Na_2CO_3 is more highly responsive to central stimulation of the saphenous nerve.

But the prolonged apnea (at 8.6 per cent O_2) was eventually broken in the absence of any known change of peripheral stimuli. Control experiments showed that the renewed breathing cannot be alternately explained

by an accidental incomplete block permitting conduction of chemoceptive signals at the peak of a heightened carotid body discharge, because the same type of renewed breathing occurred after bilateral distal section of the sinus nerves during the apnea of cold block. Provided unknown reflexogenic stimulation from sources other than the carotid and aortic bodies can be disregarded, the renewed breathing must be considered of centrogenic origin. For the present, the nature of the stimulus reinitiating breathing can only be conjectured by a process of elimination. Had the apnea been caused entirely by a paralyzing action, that action would have been expected to increase and to have terminated in death. Had the stimulation of breathing been one of *direct* action of O_2 lack, there should have been supernormal rather than subnormal centrogenic breathing when cold block took effect. But if the apnea was due to acapnia, time was essential for a reaccumulation of acid and a rebuilding of the central stimulus. We suggest that this occurred partly as a result of the high anaerobic acid metabolism in the brain and partly as an effect of the reaccumulating acid in the blood.

For completeness it must be mentioned that apneas frequently did terminate in death without outward signs of respiratory stimulation. It is, therefore, reasonable to assume that depression capable of completely counteracting stimulation can and does occur. Signs of such depression are visible in the falling blood pressure during the last two nerve blocks of figure 3.

DISCUSSION. *The summation of centrogenic and reflexogenic breathing.* One point seems clear from the experimental findings of other laboratories, and those which we have described. Eupneic breathing in anesthetized animals is a sum of the two respiratory components—centrogenic breathing plus reflexogenic breathing. But, so far as we are aware, there has been no attempt to establish a mechanism by which they are combined. Some common denominator must, therefore, be found to account for the complementary action between centrogenic and reflexogenic breathing. The inherent forces arising in the neuron proper and those forces arising from the impingement of signals at the synapse must in some way be combined.

The electrotonic theory of nerve cell discharge and *synaptic drive* (Gesell, 1939, 1940) lends itself to such speculation and offers a relatively simple schema. (See figs. 4 and 5.) Due to a steep metabolic gradient between the dendrites and the axon hillock, estimated as 10 to 1, (Holmes, 1932) an electrotonic current is conceived to flow within the cell from the dendrites to the axon hillock. Because of the high lineal resistance of the neuraxon, the current is deflected at the axon hillock where it leaves the cell body, to return in the immediate external environment, back to the dendrites. On leaving the axon hillock, it is thought to fire this structure

at a frequency proportional to the intensity of the electronic current. Metabolic physico-chemical fluctuations, such as result from changes in O_2 and CO_2, are thought in turn to modify the intensity of this current. Changing intensity of and changing response to the electrotonic current would thus represent our so called "centrogenic component" of respiratory control.

Each of these cells (probably the reticular cells of the medulla) is covered with a dense layer of hundreds or thousands of synapses, delivering signals from all quarters, including the chemoceptors. Each signal, regardless of its origin, is thought to produce a local negativity at its point

Figs. 4 and 5. Schema showing a nervous mechanism for the summation of centrogenic and reflexogenic breathing.

of impingement and thereby increase the potential drop of the receiving neuron.[4] The intensity of the reflexogenic drive (or the reflexogenic component of breathing) is accordingly determined by the sum total of signals arriving per unit of time. Complementary action of the centrogenic and reflexogenic components thus becomes a simple matter of the addition or subtraction of one current to or from the other. The interaction of this dual mechanism of nerve cell activation allows not only a change in the sum total of centrogenic and reflexogenic components but gross differences in the relative proportions. At one extreme in which

[4] This hypothetical negativity may conceivably arise from either a specific activation or from an increased dendritic metabolism, initiated by a local electrical discharge or a chemical deposition at the synapse. Both could increase the metabolic or potential gradient and thereby the nerve cell discharge. The fact that breathing diminished gradually during the course of a continued Hering nerve block (see the lower record of fig. 1 and all of the records of fig. 3) suggests that synaptic effects long outlast the moment of their initiation. This might be regarded as a new interpretation of the general phenomenon of "after discharge." More specifically the results suggest that chemoceptor signals help to maintain the respiratory neurons at a higher degree of reactivity.

central apnea is produced by excessive pulmonary ventilation during oxygen deficiency, the central neurons would still retain their ability to respond to increasing reflexogenic electrotonic current even though the centrogenic electrotonic current is weakening. And this condition can shift to the other extreme of hypercapnia in which centrogenic breathing continues to increase long after reflexogenic breathing is abolished (see fig. 6).

The existence of two mechanisms of respiratory control, one central and the other peripheral, carries most interesting implications. Since both mechanisms seem to react to the common stimulus of cH, both may be expected to participate in the control of CO_2 and O_2 pressures in the

CENTROGENIC AND REFLEXOGENIC
RESPIRATORY COMPONENTS
DURING MODIFIED BREATHING
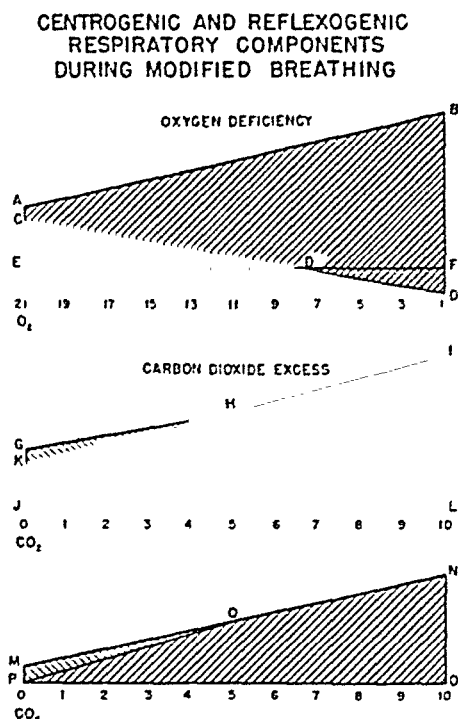
Fig. 6. Schema showing the oppositely changing proportions of centrogenic and reflexogenic breathing in progressively increasing hypoöxia and hypercapnia.

body. Nevertheless hyperpnea, caused either by oxygen scarcity, or carbon dioxide excess, tends to become exclusively reflexogenic or centrogenic. In other words, one mechanism gains the upper hand of the other and maintains primary control. This paradoxical situation, we believe, is explainable with the aid of the reaction theory. During O_2 lack the chemoceptors by virtue of a disproportionately high reactivity to changes in their own acid metabolism (Winder, 1937; Bernthal, 1938; Bernthal and Weeks, 1939; Winder, Bernthal and Weeks, 1938; von Euler, Liljestrand and Zotterman, 1939) gain the advantage and give increasing predominance to reflexogenic breathing as scarcity of oxygen grows. As

a result of increasing ventilation and of the other alkalinizing influences, centrogenic breathing is diminished. We have attempted to indicate these trends of centrogenic and reflexogenic breathing under theoretically ideal conditions in figure 6 in which so called central paralysis is missing. Both types of breathing are plotted on the ordinates against oxygen percentage of the inspired air on the abscissas. The solid black area ECD represents the centrogenic component, rapidly diminishing as a result of increasing hypocapnia, and the cross hatched area $ABFD'DC$, the more rapidly increasing reflexogenic component, possibly potentiated by synaptic alkalinization. The stippled area DFD' indicates diminution of the subliminal centrogenic component.

According to this conception the chemoceptors are not fully protected against increasing acidity by the increased ventilation which they set up. They alone withstand increased acidity and thus guard the more delicate central nervous system. This will explain why it was unreasonable to expect an increased amount of lactic acid in the circulating blood of an individual exposed to low O_2 pressures. The amount of lactic acid contributed to the circulating blood by approximately one millionth of the body could not possibly be detected.

The diametrically opposite changes in centrogenic and reflexogenic breathing during progressive hypercapnia must have some deep rooted significance (see two lower schema of fig. 6). GJ represents the volume of eupneic breathing of which KJ is the centrogenic fraction produced by the stimulating action of CO_2 and KG the reflexogenic fraction. In agreement with the linear relation of the discharge frequency of the carotid body to the prevailing CO_2 pressures (von Euler, Liljestrand and Zotterman) we may assume an hypothetical reflexogenic breathing increasing along the gradient MN of the lower graph. The area $MNOP$ would accordingly represent the theoretical increase of reflexogenic breathing with increasing hypercapnia. The actual amount of reflexogenic breathing, however, is represented by area MQP or GHK above. It is, therefore, proposed that most of the reflexogenic component $PQNO$ is obliterated by a central action of CO_2, possibly by a blocking action at the synapse. As this obliteration progresses, direct central stimulation replaces that lost from the chemoceptors. Whether KI (centrogenic increase) runs more steeply than MN (reflexogenic increase) has not been determined. These graphs are of course schematic. However, one cannot avoid the question at this point, why teleologically the center takes complete control against CO_2 excesses when the chemoceptors take complete responsibility during oxygen deficiencies. The evolutionary forces which were responsible for this unique arrangement can only be conjectured. The brain is well known to require a uniformly abundant supply of oxygen while on the other hand it tolerates high pressures of CO_2 with relative impunity.

Outlying protection against the development of central oxygen deficiency is, therefore, useful. On the other hand the weakening of respiratory reflexes by a general hypercapnia may have been the issue forcing the evolution of a centrogenic mechanism of control against CO_2 excesses. A flood of carbon dioxide liberated in combat might otherwise have put an end to pulmonary ventilation when it was needed most.

SUMMARY AND CONCLUSIONS

Repeated withdrawal of known chemo-reflex support to the respiratory center (bilateral reversible cold blocking of Hering's nerve after double vagotomy and permanent sinus collapse in chloralosed dogs) during various respiratory states yielded data and conclusions as follows.

During eupneic breathing of atmospheric air or O_2 rich air, chemoceptive nerve block usually reduced the volume of pulmonary ventilation. The reduction was smaller with an O_2 rich mixture than with a mixture containing but slightly less O_2 than room air. Reasons were presented for concluding that both CO_2 and O_2 pressures prevailing during eupnea are sources of reflexogenic respiratory support.

Apnea produced by overventilation with room air was markedly prolonged by chemoceptive nerve block. This effect was much greater than the reduction of breathing by chemoceptive block during eupnea. It was concluded that the chemoceptors exert an important tonic stimulation of breathing and that they are particularly responsive to oxygen lack occurring at the end of apnea.

Repeated withdrawal of reflexogenic support during progressive hypercapnia caused a diminishing absolute reduction in breathing which disappeared at 5 to 6 per cent CO_2 in the inspired air. It was concluded that hyperpnea of high grade hypercapnia is purely centrogenic.

The peculiar absence of reflexogenic stimulation could not be explained by central paralysis, for pulmonary ventilation continued to increase with increasing CO_2 well above the 6 per cent level.

In view of the linear relation of chemoceptor discharge to CO_2 pressure, and of the progressively increasing centrogenic activity in these experiments, it is concluded that increasing CO_2 exerts an increasing central blocking action on the signals which it sets up in the chemoceptors.

Conversely, diminishing pressures are thought to diminish the central blocking action of CO_2 and, thereby, potentiate the signals arising in the chemoceptors. This relationship will explain the stimulating action of low CO_2 pressures obtaining during eupnea, and at the end of experimental apneas.

Repeated chemoceptive nerve blocks during progressively increasing hypoöxic hyperpnea produced progressively increasing reduction of breathing, vigorous breathing being finally converted to apnea. It is

concluded that hyperpnea of high grade O_2 deficiency is purely reflexogenic.

In view of the abrupt resumption of hyperpnea on chemoceptive deblock and of the suddenly increased frequency of breathing on vagal deblock, it is concluded that central depression or paralysis was but a minor factor in the reduction of the centrogenic component and that progressive hypocapnea and alkalinization from several causes was a major factor in the diminishing centrogenic component. It is further proposed that the progressively increasing hypocapnia leads to a progressively increasing potentiation of the reflexogenic signals thereby assuring an increasing dominance of the reflexogenic component.

Prolonged apneas resulting from maintained withdrawal of chemoceptive support during hypoöxic hyperpnea, frequently gave way to renewed breathing. This was attributed to reaccumulation within the center of acid derived from its own acid metabolism and to increasing acidemia.

Granting that a localized acidity of the chemoceptors is the stimulating influence producing a general alkalinization of the body during hypoöxia, the basic physiological chemical control of breathing (acid excess and O_2 deficiency) is again broadly interpretable in terms of the reaction theory. Not only is the activity of the center and of the chemoceptors explained but the changing relations of centrogenic and reflexogenic breathing during varying intensities of hypercapnia and oxygen deficiency are accounted for as well.

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THE EFFECTS OF EPINEPHRINE, POTASSIUM, PENTOBARBITAL AND INSULIN ON THE CONCENTRATION OF AMINO ACID NITROGEN IN THE BLOOD OF FASTING DOGS

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Many contradictory reports (1) concerning the effects of epinephrine on nitrogen metabolism have appeared; the most general finding is an increased nitrogen output following epinephrine administration. That this increase may be this result of increased deaminization is suggested by the findings of Luck and Morse (2) and Davis and Van Winkle (3), who report an hypoaminoacidemia after epinephrine administration in rabbits. It was desired, before studying the action of other agents which might themselves stimulate epinephrine secretion, to test this latter finding in the dog.

Because there have recently been a number of papers suggesting a relationship between the actions of potassium salts and epinephrine, most of them based on the circulatory effects of these two agents, we felt that a study of their possible similarity of action on the blood amino acid nitrogen level might afford an interesting biochemical check. On the one hand stands the contention of Camp and Higgins (6) that potassium is responsible for the effects of epinephrine, and that epinephrine functions only to regulate the distribution of potassium. The experimental basis of this contention, a similarity in the effects of the two agents on the circulatory system and smooth muscle, is confirmed by Mathison, McGuigan and Higgins (7) but denied by Hug (8). That epinephrine, in common with many other agents, does raise the serum potassium level by rapid mobilization of potassium from the lever is amply demonstrated (9), although the evidence offered by Larson and Brewer that the increase in arterial pressure caused by epinephrine is not dependent on a rise in the blood potassium level weakens considerably the postulate of Camp and Higgins.

On the other hand there is the statement of Hazard (10), supported by Katz and Katz (11) and by Hug (12), that potassium causes a secretion of epinephrine, which is in turn responsible for the observed effects of potassium. The reports of Brown and Feldberg (13) and of Feldberg and Guimarais (14) that potassium liberates acetylcholine from pre-ganglionic

fibers and is itself a direct stimulant to post-ganglionic fibers are compatible with this view, as are the findings of Hazard (10) and of Silvette and Britton (15) that potassium produces an hyperglycemia. The latter finding is opposed by Kylin (16), who reports a marked hypoglycemia after potassium as well as a weakening of the pressor action of epinephrine after potassium.

If either of these contentions is to stand in the face of biochemical evidence, it is necessary to discover whether the actions of epinephrine and potassium salts on the blood amino acid nitrogen level are similar.

EXPERIMENTAL. We attempted to investigate this by the use of healthy adult male mongrel dogs, weighing between ten and twenty kilograms, which were trained to lie quietly while blood samples of two and one-half cubic centimeters each were withdrawn from the saphenous vein, usually at hourly intervals during the experimental period. The dogs were fasted forty-five to forty-eight hours prior to the beginning of the experiment, and in general dogs were used in rotation so that each dog was employed only once a week. Determinations of amino acid nitrogen were made by the method of Danielson (17), modified only by the replacement of the colorimeter by a photelometer (Central Scientific Company) with a suitable filter (Cenco, no. 1, blue).

Fasting unanesthetized dogs. A series of fasting controls was run under the same experimental conditions as the other series in order to rule out the effects of fasting, hemorrhage (as reported by Bischoff and Long (20) and by Luck and Morse (2) as a cause of hypoaminoacidemia), and all manipulations involved in the experimental procedure, especially those which might cause apprehension and subsequent epinephrine secretion on the part of the dog.

Five experiments on four dogs constitute the series. A slight fall in amino acid level (0.7 ± 0.2 mgm. per cent) was noted, the significance of which when tested by the use of Fisher's "t" and probability tables (18) proved to be so small as not to appear on the tables.

Pentobarbital. A series of anesthetized control animals seemed desirable for two reasons: first, that an anesthetic would be necessary later during the administration of the highly irritant potassium; and second, that as many of the reports upon which contradictory conclusions are based mention the use of an anesthetic but do not rule out the possible action of the anesthetic, such a study might shed some light on the cause of disagreement.

Pentobarbital sodium was administered intravenously (in a dose of 27 mgm. per kgm. body weight) immediately after the pre-injection sample was withdrawn. Nine experiments were run, using five different dogs. The mean of the maximum falls in amino acid concentration after pentobarbital was 1.7 ± 0.3 mgm. per cent; when this is compared with the

corresponding value in the control series, the probability that such a difference could occur solely through errors of random sampling is about five in 100 (P = 0.0544): one can thus say with fair assurance that pentobarbital has caused a significantly greater lowering of the blood amino acid nitrogen than sampling or fasting alone.

Epinephrine. In the first epinephrine series, that on unanesthetized dogs and consisting of six experiments on five dogs, epinephrine hydrochloride was administered subcutaneously immediately after the pre-injection blood sample had been withdrawn. The dose used, 0.1 mgm. per kgm., always produced overt signs of epinephrine activity in the dogs. When the mean lowest post-epinephrine value is compared with the pre-injection mean, a lowering of 2.9 mgm. per cent is revealed, with a probability that this lowering is due to chance of less than two in 100 (P = 0.0194). When the mean of the maximum falls produced by epinephrine (2.9 ± 0.8 mgm. per cent) is compared with the corresponding value in the control series, we find again the chances that such a difference could occur by random sampling errors are only three in 100 (P = 0.0296). It is clear that epinephrine produces a marked lowering of the blood amino acid nitrogen level in unanesthetized dogs, a lowering significantly greater than that observed in the control series. This finding confirms the reports of previous workers and extends them to a different species, the dog.

In the second epinephrine series, introduced to afford a direct check on the possible mutual interaction of epinephrine and pentobarbital and to provide a more logical basis for comparison with the potassium studies, pentobarbital sodium, 27 mgm. per kgm. body weight, was administered intravenously to four dogs immediately after the pre-injection sample; a post-pentobarbital sample was drawn one hour later, and epinephrine hydrochloride, 0.1 mgm. per kgm. body weight, administered subcutaneously immediately after this sample. Again a slight lowering of the amino acid nitrogen level was observed. Comparison of the mean of the maximum falls in this series (1.8 ± 0.4 mgm. per cent) with like values in the three preceding series reveals that while this fall is significantly greater than that seen in the fasting control animals (P = 0.0442), it does not differ at all from that produced by pentobarbital alone (P = 0.6560), and is less in degree, though not significantly so, than that produced by epinephrine alone (P = 0.2554). It is interesting to compare this finding with that of Hrubetz and Blackberg (19) who report that pentobarbital impairs also the response of the blood sugar to epinephrine.

Potassium chloride. Here, as in the epinephrine group above, pentobarbital sodium, 27 mgm. per kgm., was administered intravenously immediately after the pre-injection sample. Post-pentobarbital samples were drawn hourly and potassium chloride 1.1 per cent (isotonic) was then run into the vein at such a rate that the animal received 1.5 mgm.

KCl per kgm. per minute for one hour, or a total of 90 mgm. per kgm.; samples were drawn when possible during the potassium infusion, immediately after cessation, and at hourly intervals thereafter. The series consisted of six experiments on four dogs.

A very slight fall in amino acid nitrogen was noted, the mean value at the end of the potassium infusion being 1 mgm. per cent lower and that one hour after the infusion 1.4 mgm. per cent lower than the mean preinjection value; in both cases the probability that such a difference might be equalled or exceeded through errors of random sampling is as high as one in four. When the average maximum drop (1.4 ± 0.4 mgm. per cent) is compared with that in the control series, the probability of such a difference as a result of random sampling errors is almost one in five. Potassium chloride did not, in the doses used and under the conditions of our experiments, cause a significant lowering of the blood amino acid nitrogen level of fasting anesthetized dogs.

TABLE 1

Summary of results

	MEAN LOWERING OF AMINO ACID CONCENTRATION	PER CENT OF PREINJECTION	"P" VALUE VERSUS CONTROL
	mgm. per cent		
Fasting control........	0.7 ±0.2	9	
Pentobarbital......................	1.7 ±0.3	21	0.0554
Epinephrine.......................	2.9 ±0.8	34	0.0296
Epinephrine-pentobarbital..	1.8 ±0.4	24	0.0442
Potassium chloride-pentobarbital.....	1.4 ±0.4	17	0.1890
Insulin............................	2.5 ±0.3	30	0.0008

Because we felt that the failure of potassium in our experiments to cause a lowering of the blood amino acid nitrogen concentration might be the result simply of a sub-threshold dose, one acute experiment was run in which potassium chloride was injected to the point of cardiac arrest; no further change was noted in amino acid nitrogen concentration. We have thus failed to find biochemical evidence, using as our criterion the behavior of the blood amino acid level, that potassium is responsible for the effects of epinephrine or, on the other hand, that it causes marked epinephrine secretion, since we have shown that even a dose large enough to produce cardiac arrest does not significantly lower the concentration of blood amino acid nitrogen. Admittedly, some qualification of this statement is necessary, since the response to epinephrine was likewise impaired by pentobarbital. A final answer to the problem could be given if it were possible to administer potassium chloride to the unanesthetized animal, for in comparable experiments with epinephrine the results were unequivocal,—a

substantial fall in the amino acid nitrogen content of the blood was obtained invariably.

Insulin. In the course of further studies, not here reported, it seemed advisable to confirm previous reports (3, 4, 5) that insulin also produces a lowering in the concentration of amino acid nitrogen in the blood. The animals in this group, four in number, received insulin, 0.5 unit per kgm. body weight, immediately after the drawing of the pre-injection blood sample. The mean lowest post-insulin values were 2.5 mgm. per cent below the mean pre-injection values, and the statistical significance of this fall is unquestionable (P = 0.0316). If the mean of the maximum falls in this series (2.5 ± 0.3 mgm. per cent) be compared with that of the fasting controls, there remains no question that insulin produces a significantly greater lowering than fasting and sampling alone (P = 0.0008). The results are summarized in table 1.

SUMMARY

1. The effects of epinephrine, pentobarbital sodium, potassium chloride, and insulin on the fasting blood amino acid nitrogen level of dogs have been studied.

2. It has been shown that both epinephrine and insulin produce a marked hypoaminoacidemia in unanesthetized fasting dogs.

3. Pentobarbital sodium, in anesthetic doses, has been shown to cause a significant though not a marked fall in blood amino acid nitrogen concentration.

4. However, when epinephrine was administered to dogs anesthetized with pentobarbital sodium, the resultant fall in blood amino acid nitrogen concentration was no greater than that produced by pentobarbital alone.

5. The intravenous infusion of potassium chloride, 1.5 mgm. per kgm. per minute for one hour, in dogs anesthetized with pentobarbital sodium, produced no significant effect on the level of blood amino acid nitrogen.

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DISTINCTION BETWEEN ARTERIAL, VENOUS AND FLOW COMPONENTS IN PHOTOELECTRIC PLETHYSMOGRAPHY IN MAN[1]

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This paper proposes to explore the possibility of distinguishing "active" from "passive" components, of separating arterial from venous reactions, in photoelectric plethysmograms of the human skin. Such distinctions appear possible through studies of the changes in blood content, in the volume pulse and in blood flow (calculated in arbitrary units) when these changes are recorded with photoelectric plethysmographs in the manner described below.

The possibility that these objectives might be served was suggested by several considerations. The excellent correlation found by Burton (1) between the amplitude of the mechanically recorded volume pulse and the blood flow in 'the finger indicated the desirability of studying more carefully the volume pulse in various skin areas in relation to the plethysmogram of the same area. Also, the use of the volume pulse as a measure of the abundance of the arterial blood supply in various skin areas (2) implied that it might serve as an indicator of arterial reactions in skin areas which can be profitably explored with the photoelectric plethysmograph. Variations in the amplitude of the volume pulse have received some attention as qualitative criteria of arterial reactions (1), (3), (5), but a more systematic exploration of this point seemed desirable from the viewpoint of the analysis of the vascular mechanisms involved in specific plethysmograms.

If the arterial reactions could be separated out from the plethysmogram with sufficient clarity, it seemed theoretically possible, under suitable circumstances, also to distinguish simultaneously the effects of arterial inflow, venous congestion and even of venous tone in the plethysmogram. The observations reported here show considerable promise in this direction.

METHODS. The experiments described below employ the photoelectric

plethysmographs previously reported (2). When these are used in connection with resistance coupled amplifiers (4), the resulting records show the changes in blood volume and also in volume pulse amplitude. Careful inspection of the latter is most tedious. It is more practicable to record the volume pulses separately on a constant base line by employing a capacity-coupled amplifier. This permits higher amplification of the volume pulse and a correspondingly more legible record of it. This technique is equivalent to placing a leak sufficiently large in a mechanical plethysmograph to prevent changes in base line but not so large as to prevent adequate recording of the oscillations due to the pulse.

A convenient circuit for this purpose is shown in figure 1. Records taken with this amplifier and compared with those taken simultaneously
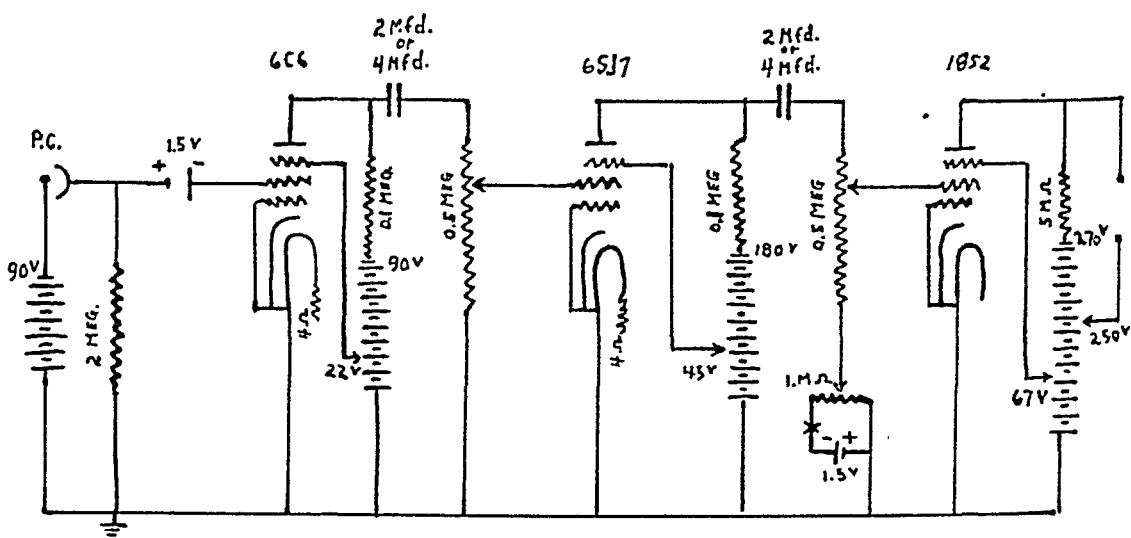
Fig. 1. Amplifier for recording the photoelectrically detected volume pulse on a constant base line. P. C.: photoelectric cell in plethysmograph. Tubes: 6C6, 6SJ7 and 1852.

from the same skin area with the resistance coupled amplifier (4), accurately show the amplitude of the volume pulse undistorted by changes in the position of the base line, when condensers of 2 mfd are used (fig. 2). Substituting condensers of 4 mfd provides for accurate recording of the wave form of the volume pulse but introduces minor variations in the base line which are of no importance in studying the wave form of the volume pulse, but which do interfere with following the variations in the amplitude of the wave. It should be noted that attempts to quantitate the volume pulse so recorded in terms of arbitrary but reproducible units (2) were not successful. True, calibration of the amplifier with standard input voltages is simple but irrelevant for the problem of the blood equivalent of the photoelectrically recorded volume pulse. Attempts to use the

"filter" technique for calibrating the volume pulse (2) ran into difficulties due to the discharge characteristics of the condensers and so were abandoned. This amplifier is not suitable for measuring the arterial blood supply of the skin.

One will observe that during constriction (fig. 2) the amplitude of the volume pulse decreases, the base of the recorded wave shifts upwards while the crest of the wave falls. This apparent shift in the base line is due to the fact that the pulse wave produces an oscillation either side of the mean which is the base line. When constriction occurs, the amplitude of the oscillation decreases in both directions toward the mean which lies nearer the base of the wave than its crest. As a result, the volume pulse so recorded mimics graphically the variations in artery diameter. This is extremely useful to the rapid inspection of arterial reactions.

Distinction of the arterial component in the plethysmogram. Simultaneous registration of the finger pad volume and of the finger pad volume pulse
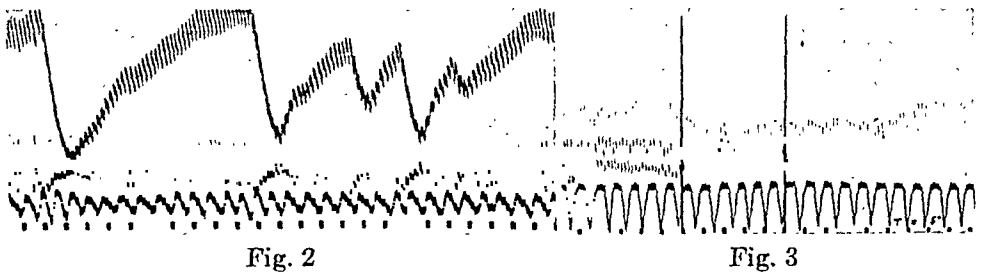
Fig. 2   Fig. 3

Fig. 2. Finger pad plethysmogram (upper record), finger pad volume pulse. Respiration, time: 5 seconds. Spontaneous waves.

Fig. 3. Finger pad volume pulse. Respiration (re-traced). Time: 5 seconds. Normal subject. Inflation of arm-cuff at first signal, deflation at second signal.

as two separate records usually shows an excellent qualitative correlation when spontaneous waves (fig. 2), and the vasomotor responses to psychic stimuli, loud noises, a deep breath, and the cold pressor test are observed. The changes in volume are, in such responses, generally proportional to the changes in the amplitude of the volume pulse and they would therefore seem to be the result of altered flow due to the reactions of the controlling arteries. Hence, it is usually sufficient, and in practice more convenient, when desiring information only on the arterial reactions, to record the volume pulse alone.

An interesting application of this technique for separating out the arterial component in the plethysmogram is supplied in the case of a medical student who had developed a psychosis with respect to his own blood pressure. Every attempt to determine brachial arterial blood pressure on him with the cuff method elicited marked sympathetic excitement: increased heart rate, rise in blood pressure, sweating (particularly on the hands). Repeated physical examination of him for the purpose of com-

missioning in the medical reserve corps served to emphasize to him the fact that he was hypertensive. Since the normal subject responds with arterial constriction in the finger to inflating the arm cuff on the opposite arm (fig. 3), it seemed of interest to compare this man's reaction (fig. 4) with that of the normal subject. The negligible constriction in his fingers at the height of the reaction when his blood pressure reading was 160/80, contrasts strikingly with that occurring in the normal subject, suggesting that his hypertension was due to the cardiac acceleration. The contours of his arterial and digital pulse waves did not demonstrate arterial disease. We therefore felt that arterial disease was not the reason for the hypertension observed every time his blood pressure was measured. It is interesting that such disturbing sympathetic excitement may be present without much influence on the digital arteries since ordinarily they are exquisitely sensitive indicators of vasomotor reflexes and are probably the first ones to respond. The understandable reluctance of this subject to present himself for vascular studies which cause him real mental trauma

Fig. 4. Finger pad volume pulse. Respiration (re-traced). Time: 5 seconds. Subject has psychosis with respect to his blood pressure. Application of cuff to opposite arm at first signal, inflation at second, deflation at third signal. B. P.: 160/80.

has prevented further exploration of his case. It is therefore recorded here in its present unsatisfactory status.

There is usually a slight delay in the changes in volume with respect to the volume pulse (fig. 2). One gains the impression that this is related to the venous capacity of the pad and that the length of this delay is proportional to the venous capacity. The increase in transparency of the pad on pressing the blood out of the pad and preventing further inflow with a Gärtner capsule, varies considerably with different normal individuals. So also the decrease in transparency due to increased blood content of the pad on blocking the venous outflow shows marked differences in various normal subjects. The results with both of these procedures seem to correlate with the lag between the changes in volume pulse and volume. The difficulties inherent in securing quantitatively comparable measurements of the skin blood content with the photoelectric plethysmograph (2) have made further quantitative comparisons in this direction seem inadvisable at present.

The changes in volume with small spontaneous waves are relatively less than the changes in volume pulse at high rates of flow. This is reasonable since engorgement of the venous plexus by high flow would tend to maintain the volume constant despite oscillations in arterial tone which would exert their primary effect on the volume pulse.

Distinction of flow components in the plethysmogram. Although the blood flow through the finger is determined ordinarily by the finger artery tone, variation in the finger blood flow may result, independently of arterial tone, from changes in blood pressure and heart action. If artery tone is unchanged, the amplitude of the volume pulse would remain constant, while the blood content of the venous plexus would increase with an increased inflow due to increased heart rate. An occasional response in the finger pad to inhalation of amyl nitrite illustrates this relation (fig. 5). Finger pad volume increases for 35 seconds before greater am-
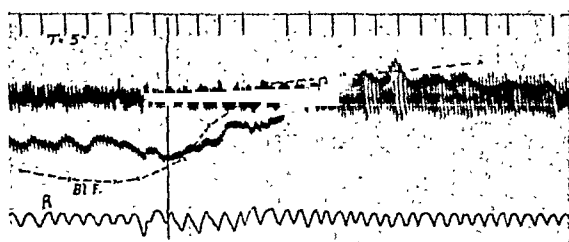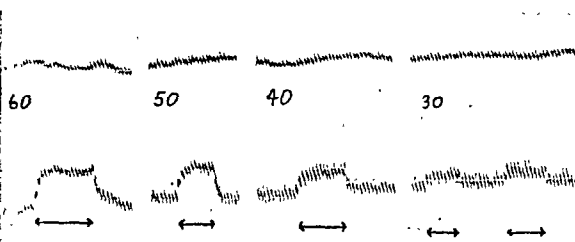
Fig. 5   Fig. 6

Fig. 5. Finger pad volume pulse (upper record), finger pad plethysmogram (lower record). Time: 5 seconds. Respiration (re-traced). Bl. F.: Plot of product, volume pulse × heart rate, in arbitrary units. Amyl nitrite at vertical signal.

Fig. 6. Plethysmograms of neighboring finger pads. Gärtner capsule on proximal phalanx of finger giving lower plethysmogram. Pressures in capsule: 60, 50, 40, 30 mm. Hg in that order.

plitude of the volume pulse indicates onset of arterial relaxation. The rise in finger volume during this period tends to parallel the plot of the product of the heart rate and volume pulse amplitude, although there may be other components in this reaction as will be pointed out below. The maximum in the finger volume is reached when the value of this product is greatest. The value of the product, heart rate x volume pulse amplitude, seems to be a numerical expression in arbitrary units of the arterial flow. Although the validity of this relation awaits direct experimental verification with blood flow methods, the assumption of its correctness makes intelligible certain plethysmographic data such as are illustrated in figure 5.

It should be pointed out that failure in the correlation between changes in the volume pulse, in volume, and in flow, is observed only infrequently in the finger pad where variations in arterial tone appear to dominate blood flow and blood content. It is only under exceptional circumstances,

as illustrated in figure 5, that variations in flow may occur here independent of constancy in arterial tone with corresponding effects on volume. However, in the case of head skin where changes in arterial tone seem to be exceptional, the correlation between volume pulse, volume and flow is poor, due apparently to the operation of certain factors on the venous side of the circulation here.

Distinction of venous components in the plethysmogram. The very great physical difficulties involved in recording changes in venous tone have made us watch with especial care for any indication of varying venous tone in our plethysmograms. Theoretically, an increase in the blood content of the observed vascular bed independent of changes in arterial tone and in arterial inflow, or of mechanical factors on the venous side of the circu-
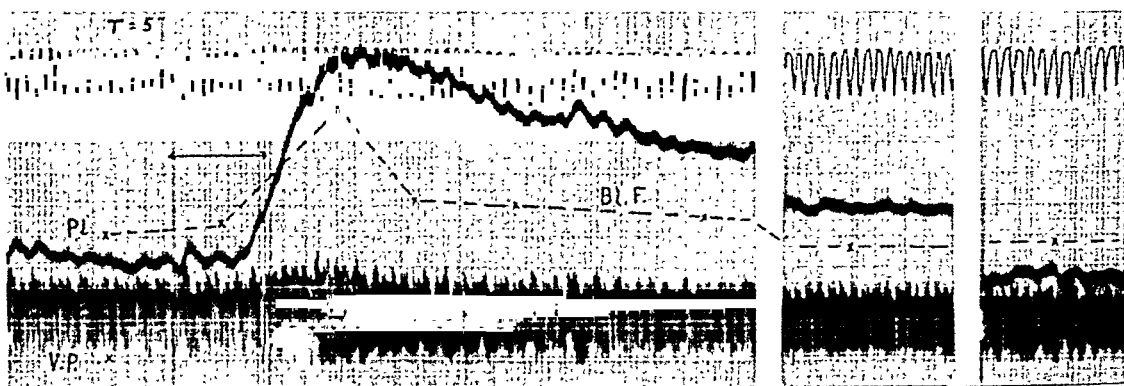
Fig. 7. Forehead plethysmogram and forehead volume pulse (lower record). Time: 5 seconds. Respiration (retraced). Bl. F.: Plot of product, volume pulse × heart rate, in arbitrary units. Ten minutes between first and second sections. Seven minutes between second and third sections. Amyl nitrite between signals.

lation, might be taken as evidence of dilatation of capillaries or veins or both.

The operation of mechanical factors such as obstruction to venous return and changes in intra-thoracic pressure, is usually evident in the plethysmogram and requires no illustration. It is interesting to note, however, that the blood content of the head skin varies much more readily with the intra-thoracic pressure than does that of the finger. Respiratory waves are more marked in the forehead plethysmogram. Effects on the venous drainage from swallowing, coughing, or a deep breath, are very obvious in the forehead, ear, and nose, although usually absent in the finger pad. The ease with which the venous blood content of head skin is altered is a complication in the study of arterial reactions in this area since the changes in volume may or may not be related to variations in arterial tone. We have been unable to decide whether these obvious differences in the behaviour of the venous drainages of the finger and

head skin are due to differences in the pressure in and extent and capacity
of the venous plexus or to differences in venous tone. Engorgement of the
small veins of the finger pad by obstructing venous outflow from the finger
with a Gärtner capsule requires surprisingly high pressures. Values as
high as 30 to 40 mm. Hg in the capsule are required in normal subjects
to produce a detectable increase in blood content of the pad (fig. 6). This
poses the interesting suggestion that these capsule pressures may measure
the venous tone in the pad.

More direct indication of changes in venous or capillary tone is occa-
sionally offered in the exceptional reactions to amyl nitrite inhalation
(fig. 7). In this instance, arterial dilatation as indicated by the volume
pulse occurred at the same time as the volume increased. The maxima
are reached very nearly simultaneously in both curves, but recovery
is strikingly different in the two curves. The volume pulse returned to
the pre-administrational level in ninety seconds, but the volume required
nineteen minutes for return. The plot of the product, volume pulse x
heart rate, shows that this delay was not due simply to increased flow but
argues for a temporary loss of tone on the venous side of the circulation
here, thus accounting for the congestion. A similar loss in venous tone
may also have been a factor in the interpretation of figure 5, but the
parallelism between flow and volume is too close here to recognize a venous
component in the reaction.

DISCUSSION. The interpretation of these experiments is based on two
relations for which the evidence is good but still incomplete:

1. *The amplitude of the volume pulse is a direct measure of arterial tone.*
This relation seems to hold under normal circumstances (1), (2). It is a
common experience in plethysmography that the volume pulse diminishes
with arterial constriction as judged by direct inspection of the blood-
vessels, by measurement of blood flow, and by such indirect criteria as
temperature measurements. The accuracy of the relation is disturbed
by changes in the position of the limb (6) but this source of error is not
involved in the experiments reported here.

2. *The product, amplitude of volume pulse x heart rate, is proportional to
blood flow.* This relation holds with considerable precision when the
heart rate is constant, for then the product is directly proportional to the
volume pulse which in turn shows good correlation with flow (1). The
volume of the part then also correlates well with the amplitude of the
volume pulse (fig. 2). The change in volume also correlates well with the
product when both heart rate and volume pulse change and when there
is no good reason to suspect venous congestion or change in venous tone.
However, experiments are required for establishing the validity of this
relation by direct measurement of blood flow. At present, the argument
must rest on indirect evidence.

It follows from these two relations that the volume changes shown on the plethysmogram may be interpreted in terms of the individual contributions made by changes in arterial tone (under special circumstances). Thus, a plethysmogram showing increased volume and also increased volume pulse indicates arterial dilatation as a main factor in the increased flow and volume (fig. 2). If the calculated increase in flow parallels closely the increased amplitude of the volume pulse, arterial dilatation is the main component in altering the plethysmogram (fig. 2). If the calculated increase in flow parallels the product, volume pulse x heart rate, the volume pulse amplitude remaining constant, the increased flow is probably "passive" and does not involve arterial dilatation (fig. 5). Again, an increase in volume continuing after arterial dilatation and flow have returned to resting levels (fig. 7) may be due to venous congestion resulting from obstructed venous return or to loss of venous tone (fig. 7). Where there is no reason to assume obstruction to the venous return, one may infer a decrease in venous tone (fig. 7).

It is appreciated that gross changes in the dynamics of the heart beat will modify the validity of these theoretical considerations. It is appreciated that changes in the stroke of the heart may and do alter the amplitude of the volume pulse in a peripheral vascular bed such as the finger pad. But the effects of these factors are often recognizable. The cautious application of these considerations in photoelectric plethysmography in circumstances which probably do not influence heart action sufficiently to affect seriously the validity of these plethysmographic criteria, leads to useful information in the study of the circulation in the human skin.

SUMMARY

This paper studies the possibility of distinguishing "active" from "passive" components, of separating arterial from venous reactions in photoelectric plethysmograms of the human skin.

A technique is described for recording the volume pulse separate from the plethysmogram, with a photoelectric plethysmograph and capacity coupled amplifier.

The arterial component in the plethysmogram is distinguished by the amplitude of the volume pulse (figs. 2, 3, 4).

The flow component is indicated by the product, amplitude of the volume pulse x heart rate (figs. 5, 7). The value of this product appears to parallel flow.

The analysis of the volume changes recorded in the plethysmogram involves evaluating the arterial and flow components by these criteria and so by a process of exclusion, differentiating when possible the contribution of the venous component (fig. 7).

Measurement of the venous pressure in the finger pad by obstructing

the return with a Gärtner capsule indicates a surprisingly high venous pressure in the pad and also a high venous tone there (fig. 6).

Evidence is presented which seems to show that moderately heavy doses of amyl nitrite produce prolonged loss of venous tone (fig. 7).

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DO THE CAROTID SINUSES EXERT A PRESSOR ACTIVITY WHEN THE SYSTEMIC BLOOD PRESSURE IS LOW?

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In recent years much attention has been paid by many investigators to the part played by the nervous system in controlling the circulation under various conditions. As a consequence of this work discoveries have been made which are not only of physiological interest but which also shed light on a number of imperfectly understood clinical states. Outstanding within this field of research has been the work on the carotid sinus.

Hering (1) was the first to present real proof of the physiological activity of the carotid sinus. Hering (2) and later Koch (3) considered the sinus nerves as exercising solely a tonic inhibitory influence on the circulation. They believed the afferent discharge in the sinus nerves to be dependent on an adequate degree of distension of the sinus by the blood pressure. They regarded the rise of blood pressure and acceleration of heart rate on carotid occlusion as due to a decrease or abolition of sinus inhibitory action following the fall of endosinal pressure below the threshold value; they supposed that afferent impulses cease to pass up to the bulbar centers, which consequently overact. In support of this view is the fact that section of the sinus nerves or cocainization of the sinuses produces similar pressor effects.

Wright (4) does not believe it yet possible to exclude conclusively the existence in the walls of the sinuses of pressor fibers which are stimulated by the fall of pressure. Heymans and Bouckart (5) have found in perfusion experiments that when the pressure in the sinus of the dog was raised from 0 to 50 mm. of Hg, instead of getting no response as Koch (6) claims, they obtained a reflex rise in blood pressure. Wright (4) has found the same in the cat. McDowall (7) showed that after a hemorrhage, section of the vagi may produce a further fall of blood pressure, owing to the removal of the compensatory afferent pressor influence of these nerves. Stimulation of the central end of cut vagi causes an increase in the blood pressure, in some cases, which is indicative of the presence of pressor fibers. Using a technique similar to that employed by McDowall (7) I have studied

the carotid sinuses of dogs in an effort to determine the presence or absence of pressor activity in this structure.

METHOD. Large healthy dogs were used in this study. They were anesthetized with chloralose (60 to 85 mgm. per kgm. intravenously). Direct blood pressure from the femoral artery and respiration by the pneumographic method were recorded on the kymograph. The carotid sinuses were carefully dissected free and their activity determined. The results presented here are from only those animals (16 in number) which showed definite circulatory and respiratory reflex response to carotid occlusion and sinus massage. The vagi were severed to remove any compensatory

TABLE 1

DOG	WEIGHT	BEGIN-NING B.P.	PER CENT OF TOTAL BLOOD VOL. REMOVED	REMOVAL TIME	FINAL B.P.	EFFECT OF DENERVATION ON B.P.
	kgm.	mm. Hg		min.	mm. Hg	mm. Hg
1	17	140	43	70	45	Rose gradually........... 15
2	20	120	50	65	45	Rose in next 5 min........ 55
3	24	130	25	55	50	Rose gradually........... 20
4	18	145	40	55	45	Rose.................... 15
5	20	130	31	80	60	Rose in next 5 min........ 45
6	15	170	50	60	60	No change
7	26	160	35	80	55	No change
8	23	115	39	90	45	Rose in next 5 min........ 25
9	22	130	30	65	50	Rose in next 10 min....... 25
10	18	125	55	90	55	No change
11	26	100	35	100	55	Rose in next 5 min........ 30
12	20	110	35	60	55	No change
13	18	140	60	80	60	Rose gradually........... 30
14	12	150	50	70	60	Rose in next few min..... 40
15	18	145	40	50	70	No change
16	25	115	30	90	70	No change
Av.	20	133	40	72	55	30

activity by this route. The animals were bled slowly over periods varying from 50 to 100 minutes, from the other femoral artery. The bleeding was sufficient to lower the blood pressure to a sustained low level, previously selected, within the range of 45 to 70 mm. of Hg. It was felt that a pressure within this range would be sufficient stimulus to arouse pressor activity, if present. After making certain that the blood pressure was constant at the desired level the isolated carotid sinuses were rapidly denervated by mechanical stripping and painting with phenol. The latter was subsequently washed off with warm saline. All changes in blood

pressure, pulse rate or respiration in the next several minutes were read from the kymograph record.

RESULTS. In ten of the sixteen animals there was a gradual rise of blood pressure varying from 15 to 55 with a mean of 30 mm. of Hg during the next few minutes following denervation. The rise in pressure in all cases was slow and gradual rather than abrupt. Six animals showed no change in blood pressure during a similar period and in no case was there a drop in blood pressure.

There were no appreciable changes in either respiration or pulse rate referable to the denervation.

DISCUSSION. An increase in blood pressure, from a sustained low level, following denervation of the carotid sinuses indicates that functioning afferent depressor fibers were disrupted by this operation. This is contrary to Koch's (6) work for the dog, who found no depressor activity present in the sinus below about 60 mm. of Hg; neither does it substantiate the work of Heymans and Bouckart (5) who found pressor activity in the carotid sinus below 50 mm. of Hg for the dog, nor Wright's findings (4) of a similar nature for the cat. That compensatory measures could have been responsible for the rise in blood pressure in ten of the sixteen experiments does not seem probable, for the low pressures were maintained for 10 to 15 minutes previous to the denervation. Though the rise in pressure in those cases in which it rose was gradual, it was complete within one to five minutes in all but animal 9.

It is not surprising that there was no appreciable effect on pulse rate attributable to the denervation since the vagi were cut and Koch (3) has shown that the sinus reflex has relatively little effect on this factor as compared to the aortic reflex.

Respiration varied, as expected, to carotid occlusion, sinus massage and vagus section. At the low pressure most animals displayed a gasping type of respiration which in most cases was relieved at about 70 mm. of Hg in those animals whose blood pressure increased subsequent to sinus denervation. The change was not correlated with the denervation, but rather with systemic blood pressure, indicating that the gasping type of respiration was relieved by central effect rather than peripheral.

SUMMARY

Lowering of the blood pressure in dogs anesthetized with chloralose to sustained low levels varying from 45 to 70 mm. of Hg does not arouse any pressor activity in the carotid sinuses, which if present would be demonstrable by a fall in pressure when these areas were denervated. A gradual rise in pressure in 10 of the 16 experiments indicates the presence of depressor activity at the time of denervation. In the other 6 there was no change in blood pressure.

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RELATION OF THE GROUPS OF THE ADRENALIN MOLECULE TO ITS CARDIO-ACCELERATOR ACTION[1]

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Intrinsic differences in sympathetically innervated effectors were suggested by Elliott (1) to explain the excitatory effects of adrenalin on smooth muscle in certain organs and inhibitory effects on smooth muscle in other organs. With the demonstration of the adrenalin-like nature of the adrenergic mediator (2) the concept of differences in receptive mechanisms has been used to explain the effects of the mediator. The necessity for postulating two or more receptive mechanisms is evident, but the fundamental nature of these mechanisms is still unknown.

We have suggested that a possible difference in the reception of adrenalin by adrenergically innervated effector cells could be explained by assuming that certain groups of the adrenalin molecule are necessary for the effective union of adrenalin with the receptive mechanism in one type of effector, while one or more different groups unite with the receptive mechanism in another type of effector (3). It appears that this possibility may be tested by making quantitative comparisons of single-effector responses to adrenalin and to other phenylethylamine derivatives lacking one or another of the characteristic groups of the adrenalin molecule. Such a study has been completed with the denervated intestine in the unanesthetized dog (3). The following is a quantitative study of these compounds as accelerators of the denervated heart under experimental conditions identical to those used in the study of the intestine.

METHODS. At first dogs were prepared with denervated hearts by performing the series of three operations described by McIntyre (4). The right sympathetic trunk was removed from the stellate through the fifth thoracic ganglion, and the right vagus was cut below the recurrent laryngeal nerve. The corresponding part of the left sympathetic trunk was removed in a second operation. Following complete recovery from these two operations the left vagus was cut in the neck. After it was discovered that neosynephrin would produce a marked rise in blood pressure at an injection rate considerably below threshold for accelerating

the rate of the denervated heart, this compound was used to test for the presence of cardio-inhibitory fibers. When cardiac slowing could be produced by neosynephrin in dogs having had the above three operations the presence of cardio-inhibitory fibers in the right vagus could be demonstrated by direct stimulation of the peripheral end of the cut nerve. The latter animals of the series were bilaterally vagotomized in the neck following recovery from bilateral sympathectomy in the thorax. These animals all experienced difficulty in swallowing and were regularly given glucose and saline intravenously. Records were obtained within one to three weeks after the vagotomy. None of the animals showed cardiac slowing during neosynephrin injection after sympathectomy and bilateral vagotomy in the neck. Four of the dogs had their adrenals demedullated by cautery previous to the cardiac denervation.

TABLE 1

Relative potency of adrenalin and simpler phenylethylamine derivatives as accelerators of the denervated dog heart

The reciprocal of each number in the table indicates the potency of the corresponding compound relative to adrenalin

		DOG								EXTREMES
		1	2	3	4	5	6	7	8	
Adrenalin (l)........	$(OH)_2C_6H_3 \cdot CHOH \cdot CH_2NHCH_3$	1	1	1	1	1	1	1	1	1
Arterenol (dl).......	$(OH)_2C_6H_3 \cdot CHOH \cdot CH_2NH_2$	1½-2	1½-2	1½-1¾	1½-2	¾-1½	1½-2	1½-2	1-1½	¾-2
Neosynephrin (l)...	$m\text{-}(OH)C_6H_4 \cdot CHOH \cdot CH_2NHCH_3$				25	50	50	25-40	50	25-50
Epinine.............	$(OH)_2C_6H_3 \cdot CH_2 \cdot CH_2NHCH_3$				40	50	50	40-50	25-40	40-50
Synephrin (dl)	$p\text{-}(OH)C_6H_4 \cdot CHOH \cdot CH_2NHCH_3$				500-2500	500	500	1000-1500	1000	500-2500

Electrocardiographic records were taken from the denervated hearts under "near-basal" conditions during intravenous injection of the compounds from an electrically driven syringe. Details of the method have been described in a previous paper (3). The series of compounds used and the formula of each is shown in table 1. For each compound the lowest constant injection rate capable of causing a greater than five per cent acceleration of the denervated heart within less than 60 seconds was determined. Also each dog was given a series of adrenalin injections at rates from barely threshold to several times the threshold accelerator dose, with correspondingly greater acceleration, and these results were used as a scale for judging the accelerator potency of the other compounds injected at rates two to four times the threshold accelerator rate. The results below are obtained from analysis of 99 records from eight dogs.

RESULTS. *Minimal accelerator injection rate of adrenalin for denervated hearts of normal and adrenal demedullated dogs.* The minimal injection rate capable of causing more than 5 per cent acceleration of the denervated

heart in less than 60 seconds ranged from 0.00020 to 0.00057 mgm. per kilo per minute. Wada and Kanowoka (5) reported that injection of adrenalin at rates from 0.00020 to 0.00030 mgm. per kilo per minute was the minimum effective dose for accelerating the rate of the denervated heart and for increasing the blood pressure of non-anesthetized and non-fastened dogs having their adrenal glands demedullated. Dragstedt (6) has reported 0.00020 as the minimal pressor dose of adrenalin for un-anesthetized dogs. The sensitivity of the denervated Thiry fistula as an indicator for adrenalin in normal dogs is emphasized by these figures, since the denervated intestine is significantly inhibited by injection rates from 0.00010 to 0.00020 mgm. per kilo per minute.

TABLE 2

Acceleration of the denervated heart by adrenalin and simpler phenylethylamine derivatives

Dog 4, weight, 10 kilo, adrenal glands demedullated

DILUTION	CUBIC CENTIME-TERS INJECTED PER MIN.	HEART RATE PER MIN. FOR THE 15 SEC. BEFORE INJECTION	HEART RATE PER MINUTE FROM THE BEGINNING OF THE INJECTION CALCULATED FROM SUCCESSIVE 15 SEC. INTERVALS					
			1st	2nd	3rd	4th	5th	6th
1. Adrenalin..........	1:250,000	1	110	109	108	114	120	124	
2. Adrenalin..........	1:250,000	2	107	106	114	136	154	164	168
3. Adrenalin..........	1:250,000	2	112	114	118	133	140		160
4. Adrenalin..........	1:250,000	4	104	104	140	180	204		
5. Arterenol..........	1:250,000	2	117	116	123	132	134		
6. Arterenol...	1:250,000	4	110	111	125	144	154	160	162
7. Neosynephrin......	1:20,000	4	112	112	116	130	140	148	160
8. Epinine..........	1:25,000	1	108	107	107	106	106		
9. Epinine..........	1:25,000	4	104	104	104	106	110	114	
10. Synephrin........	1:1,000	2	109	108	109	112	113	115	
11. Synephrin........	1:100	2	107	106	116	142			

The minimal accelerator dose of adrenalin for the denervated heart was essentially the same for the animals with adrenals demedullated as for those with adrenals intact. Such a result would be explained if the basal output of adrenine from the adrenal glands is negligible, or if the denervated heart becomes more sensitive to adrenalin as a result of adrenal demedullation. Wada and Kanowoka (5) found that the basal rate of the denervated heart of the dog was unchanged by inactivation of the adrenal medullae.

Data from dog 4 shown in table 2 illustrate typical results obtained from continuous injection of adrenalin at low rates. An injection at the rate of 0.0004 mgm. per kilo per minute caused acceleration beginning in the third 15 second period. The rate increased steadily until 75 seconds

from the beginning of the injection when it was 14 beats per minute faster than normal. With an injection of 0.0008 mgm. per kilo per minute the acceleration began within the second 15 second period and increased almost linearly for two minutes until the rate was 50 beats per minute faster than normal.

When very low injection rates are sustained for several minutes, it is observed that acceleration of the denervated heart begins after a somewhat longer latent period than when larger doses are used, and the rate gradually increases until a level is reached which is maintained during an injection of 10 to 20 minutes' duration. For example, adrenalin was injected into dog 8 at a rate of 0.00012 mgm. per kilo per minute for 20 minutes. The normal rate was 122 beats per minute. During the 4th 15 second period after the beginning of the injection the rate per minute was 128; at the end of 3 minutes the rate was 142 and did not fluctuate significantly from this level until the end of the 20 minute period. This type of response suggests a building up of the concentration of adrenalin in blood for several minutes as the injection is continued and is difficult to explain otherwise. However, Rogoff and Marcus (7), using the "caval pocket" technique, detected no increase in the adrenalin content of the blood of the adrenal vein during continuous intravenous injection of adrenalin at rates many times as fast as those used in these experiments.

The ability of a near-threshold accelerator injection rate of adrenalin to maintain a fast rate of the denervated heart during the continued injection is to be contrasted with the failure of adrenalin to keep the intestine inhibited when injected at rates several times the threshold intestine-inhibiting dose (8). The denervated intestine becomes refractory to the inhibitory effects of adrenalin, while the denervated heart does not become refractory to the accelerator action of adrenalin. If, therefore, the cardiac rate and the intestinal motility be recorded simultaneously, an adrenalin injection rate may be used which results in both cardiac acceleration and intestinal inhibition at the beginning of the injection, while there is only cardiac acceleration at the end of the injection.

Relative potency of adrenalin and simpler phenylethylamine derivatives as accelerators of the denervated heart. Each of the four compounds, differing from adrenalin by lacking one or another of the three hydroxyl groups or the methyl group on the nitrogen atom, was capable of accelerating the denervated heart if injected in sufficient quantities. None of the compounds produced a slowing of the denervated heart. The difference in the potencies of the compounds as accelerators of the denervated heart is given in table 1. The extremes are listed for the increase in injection rates necessary to produce an acceleration comparable to that produced by a given dose of adrenalin. Therefore, the reciprocal of the number indicates the potency of the corresponding compound as compared with

adrenalin. A typical series of analyses used in determining relative potency of the compounds is shown in table 2.

The results for the denervated heart resemble those for the denervated intestine in that the greatest reduction in potency in each case is obtained by removal of the m—OH group of the adrenalin molecule, and the least reduction in potency results from removal of the—CH_3 group. In fact, since dl-arterenol (non-methylated adrenalin) was used in these studies, it can not be stated whether l-arterenol would be slightly more potent or slightly less potent than l-adrenalin. Removal of any one of the —OH groups of the adrenalin molecule results in a compound having much lower cardio-accelerator potency than adrenalin.

Since the results are concerned with compounds each lacking only one of the four groups which distinguish adrenalin from phenylethylamine, the question arises whether it may be concluded that all phenylethylamine derivatives simpler than adrenalin will be less potent. Such a generalization is not possible with regard to the pressor potency of phenylethylamine derivatives (9), but the pressor response is the result of the activity of various types of effectors and compensatory mechanisms. The generalization could not be expected to apply to anything but single-effector responses. Bacq's studies using the nictitating membrane (10) (11) indicate that tyramine, which lacks three of the groups investigated in this study, is less potent than any one of the three compounds lacking only one of these groups, and phenylethylamine is less potent than tyramine.

The minimal pressor injection rate for adrenalin in unanesthetized dogs is approximately the same as the minimal cardio-accelerator injection rate (see above). Removal of the para—OH group of the adrenalin molecule lowers the pressor potency to $\frac{1}{4}$ that of adrenalin but lowers the cardiac-accelerating potency to $\frac{1}{25}$ to $\frac{1}{50}$ that of adrenalin. Therefore, neosynephrin may be injected at a rate producing a pressor response without accelerating the denervated heart. Since adrenergic denervation in other tissues sensitizes them to neosynephrin (3), the innervated heart may be expected to be even less sensitive than the denervated heart to the direct accelerator action of neosynephrin. The only effect of neosynephrin on the rate of the innervated heart, with the low dosages, is reflex slowing as a result of the increase in blood pressure. When denervation of the heart was incomplete neosynephrin caused cardiac slowing, but in the completely denervated hearts no effect on rate was obtained. High dosages caused only acceleration of the denervated heart. Orth et al. (12) observed that neosynephrin was the only one of their series of sympathomimetic amines that did not markedly accelerate the sino-auricular rate under cyclopropane anesthesia. Also, neosynephrin was least apt to produce cardiac irregularities. When comparable pressor

dosages were used all of the compounds except neosynephrin increased the sino-auricular rate under ether anesthesia. The effects of this compound illustrate the fact that a general comparison may not always be made between the potency of adrenalin and other phenylethylamine derivatives. The comparison must be made with regard to a single type of effector. As judged by changes in potency in single effectors, removal of a single group of the adrenalin molecule may interfere to a greater degree with the reception of the compound in one type of effector than in another.

SUMMARY

The potency of adrenalin, arterenol, neosynephrin, epinine, and synephrin as accelerators of the denervated heart has been determined in normal and in adrenal demedullated unanesthetized dogs. Each of the latter four compounds differs from adrenalin by lacking one of the four groups that distinguish adrenalin from phenylethylamine.

The responses of the denervated hearts of the dogs with adrenals intact were indistinguishable from the responses of the adrenal demedullated dogs. This result with very low injection rates of adrenalin would be explained by a low resting output of adrenine from the adrenal medulla or, less likely, by a heightened sensitivity of the denervated heart to adrenalin after adrenal demedullation.

Development of refractoriness to the effects of a continuous injection of adrenalin and related compounds does not follow the same course in the sino-auricular node that it does in intestinal smooth muscle. Although the minimal effective doses are similar for the two indicators, continuous injection of adrenalin at low rates produces temporary intestinal inhibition and sustained cardiac acceleration.

Each of the compounds accelerates the denervated heart when given in concentration having any effect on the heart rate, and the degree of acceleration increases as the threshold accelerator dose is doubled or quadrupled. Removal of the—CH_3 group of the adrenalin molecule does not significantly affect the cardio-accelerator potency, but removal of any one of the three—OH groups results in a compound $\frac{1}{25}$ to $\frac{1}{25000}$ as potent as adrenalin. The most important of the four groups with regard to cardio-accelerator potency is the meta—OH group.

Removal of the para—OH group reduces the cardio-accelerator potency more than the reduction in pressor potency. Neosynephrin, given at a rate that will not alter the rate of the denervated heart, produces a rise in blood pressure. Neosynephrin, injected at this rate, serves as a test for cardiac denervation, since reflex slowing of the heart will occur if any cardiac nerves are present.

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THE EFFECT OF ADRENALIN ON CARBON DIOXIDE OUTPUT AND RESPIRATORY QUOTIENT: PROPORTIONALITY WITH DOSE

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This is the fifth and last of a series which has described the hyperglycemia (Griffith, Lockwood and Emery, 1939a), lactacidemia (ibid., b), oxygen consumption (Griffith, Emery and Lockwood, 1940a) and pulmonary ventilation (ibid., b) of chloralose-anesthetized cats during and following intravenous administration of adrenalin for 5-minute periods at carefully controlled rates from minimally effective to maximally tolerated.

The matter of final interest in connection with the metabolic effect of adrenalin is the mechanism of its calorigenic action. Attempted explanation of this has leaned heavily on the respiratory quotient and has consequently varied with the finding and interpretation relating to it, these having been about as various as its investigators (Erichson, 1936). The data to be presented here will show that dosage may be a predominant factor in this diversity; and when taken into proper consideration there is no evidence that adrenalin has an invariably specific effect on the respiratory quotient. Rather, the latter seems to reflect more closely than anything else the minute volume of pulmonary ventilation (1940b) and presumably, therefore, like it, is only unspecifically the resultant at any moment of at least blood pressure, metabolic rate and lactic acid acidosis.

PROCEDURE. These experiments being the same as those previously described, details of procedure need not be repeated. Suffice to say, respiratory exchange of these chloralose-anesthetized cats was determined by collection (for approximately 5-minute intervals) and analysis of expired air.

Usually (57 of the 84 experiments) two determinations of the "normal" resting respiratory exchange, 15 to 20 minutes apart, preceded injection. This, following immediately the second of these normals, or the single one when only one was made, was always for exactly 5 minutes and was accompanied by a collection of the expired air approximately coincident with it, thus affording information of the average, immediate effect for the 5-minute injection period. Two additional determinations were made during the subsequent half-hour recovery: one 5 to 10 and the other 25 to 30 minutes from the end of injection.

Parke-Davis adrenalin chloride was diluted with neutral, isotonic NaCl solution for injection (femoral vein) of 1 cc. per minute for 5 minutes so as to effect rates of administration in milligrams per kilo body weight per minute (number of experiments in parentheses) of: 0.00025 (10), 0.00050 (9), 0.00100 (9), 0.00200 (14), 0.00400 (13), 0.00700 (10), 0.01000 (9).

Control injections of isotonic, neutral NaCl (10 experiments) were similar in amount, rate and duration.

RESULTS. *Normal Values. Intra-individual stability.* Averages of the two normals, 15–20 minutes apart, preceding injection are: carbon dioxide output, 4.65 and 4.63 cc. per kilo per minute; respiratory quotient, 0.735 and 0.741. Thus, as confirmed also by the other functions measured in this work, the physiological condition of these animals at the time of injection was one of satisfactory stability.

Inter-individual variability. Carbon dioxide output, cubic centimeters per kilo per minute, varied in the 84 experiments from 2.7 to 7.7; mean, 4.81; standard deviation, 1.05; coefficient of variation, 21.9. This is not unlike the variability of the closely related oxygen consumption (Griffith, Emery and Lockwood, 1940a) and would seem, like it, not adventitious but an expression of the intrinsic metabolic variability of these animals; since correlation: with oxygen consumption is nearly one ($r = +0.948 \pm 0.014$); with pulmonary ventilation ($r = +0.781 \pm 0.027$), is of the same order of magnitude as the latter with oxygen consumption ($r = +0.729 \pm 0.038$); and with respiratory quotient ($r = +0.532 \pm 0.055$) is only as much greater than that of the latter and oxygen consumption ($r = +0.230 \pm 0.070$) as might normally be expected.

The average values for the various injection groups, cubic centimeters per kilo per minute (with rate of injection in parentheses) are: 5.4 (NaCl); 5.5 (0.00025); 4.6 (0.00050); 4.1 (0.00100); 4.0 (0.00200); 4.5 (0.00400); 6.1 (0.00700); 4.8 (0.01000). Injection at the lowest rate was ineffective (see below); and although the result following 0.007 mgm. per kilo per minute is slightly irregular it is not sufficiently so to affect the conclusions. The remaining average normal rates are sufficiently alike to show that extremes within each group were adequately balanced and to eliminate variable normal rate of putput as a possible determining factor in the effect of injection.

Respiratory quotient varied from 0.64 to 0.86; mean, 0.745; standard deviation, 0.048; coefficient of variation, 6.49. Twelve, or 14 per cent, of the determinations were below 0.70. In our experience this is not unlike the condition obtaining in normal, unanesthetized cats and is therefore probably not the effect of the anesthesia but characteristic of these animals in the post-absorptive condition. However, extremes within the

various injection groups (in parentheses) were adequately balanced to produce very similar average values: 0.73 (NaCl); 0.75 (0.00025); 0.73 (0.00050); 0.74 (0.00100); 0.75 (0.00200); 0.75 (0.00400); 0.76 (0.00700); 0.75 (0.01000); thus eliminating extremes of resting normal values as possibly affecting the average results to be described here.

The Effect of Control Injection of Isotonic NaCl. Averages of the 10 experiments in which isotonic NaCl was injected in amount equal to that
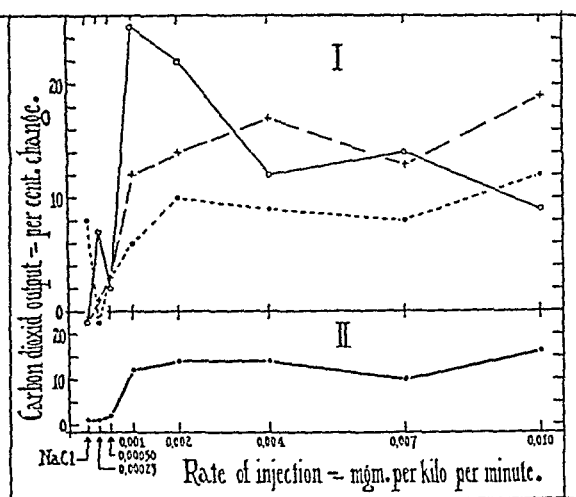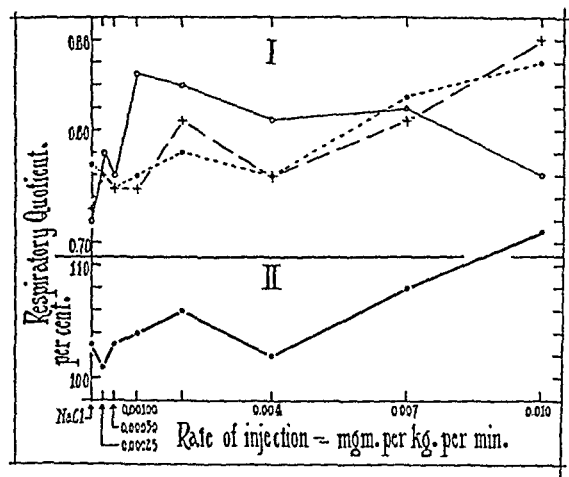
Fig 1   Fig. 2

Fig. 1. The effect upon the carbon dioxide output of chloralose-anesthetized cats of 5-minute intravenous injection of isotonic NaCl and of adrenalin administered at rates of 0.00025, 0.00050, 0.00100, 0.00200, 0.00400, 0.00700 and 0.01000 mgm. per kilo per minute.

I. The upper set of three curves: *continuous line*, values in cubic centimeters per minute for the 5-minute injection period; *dashed line*, values for the interval 5 to 10 minutes after injection; *dotted line*, values 25 to 30 minutes after injection.

II. Total summated effect for the 35-minute experimental period, including the 5-minute injection and the 30 minutes following.

Fig. 2. The effect upon respiratory quotient of the injections of isotonic NaCl and of adrenalin described in the text and the legend of figure 1. The various curves have the same significance as there described.

The three curves of part I represent the respiratory quotient in actual values; the curve of part II represents the percentage change induced by each rate of injection.

serving as vehicle for the adrenalin in the remaining experiments showed percentage values for the 5-minute injection period, and 5 to 10, and 25 to 30 minutes after, of: carbon dioxide, 99, 99 and 108; respiratory quotient, 99, 100 and 105. The high terminal result is not typical and is due entirely to 2 aberrantly high values which are quite out of keeping with the small, evenly distributed plus and minus variations of the remaining 8 experiments. These, together with the equally negative results

of the 19 additional experiments employing the two lowest rates of adrenalin injection indicate nothing disturbing evaluation of a possible adrenalin effect is inherent in the experimental procedure itself.

These control values have been used as the initial points of the curves of figures 1 and 2.

The Effect of Adrenalin Injection. Figures 1 (carbon dioxide output) and 2 (respiratory quotient) present the results in two ways: part I of each figure includes three curves depicting the results during (continuous line), and 5 to 10 (dashed line), and 25 to 30 (dotted line) minutes after injection; the single curve of part II of each figure represents the approximate total change effected by each rate of injection for the entire 35-minute experimental period as obtained by summing the products of the values for the injection period and for the intervals 5 to 10 and 25 to 30 minutes after, when multiplied by 5, 10 and 20 (minutes), respectively; thus giving an approximate summation of the area beneath each of the individual curves relating the effect of each rate of injection to time.

Threshold. With both carbon dioxide output (fig. 1) and respiratory quotient (fig. 2) there is an apparently significant increase produced during the injection period (I, continuous line) by the lowest rate of administration, 0.00025 mgm. per kilo per minute. This, however, is not sustained at the next higher rate of injection (0.00050 mgm. per kilo per minute) and the total effect for the 35-minute experimental period is in both instances so nearly nil that no considerable error can result from considering the next rate of injection, 0.001 mgm. per kilo per minute, the really minimal effective dose.

This is exactly similar to the results previously described for both oxygen consumption and pulmonary ventilation; with both of these, also, the results of injection at the two lowest rates were ambiguous, the first unequivocal increase occurring likewise with injection at the rate of 0.001 mgm. per kilo per minute. And, finally, this was also the threshold rate of injection for increase of blood lactic acid, whereas hyperglycemia was definitely evident following injection at half this rate (0.00050 mgm. per kilo per minute).

Proportionality between rate of injection and increases of carbon dioxide output and respiratory quotient. Consideration of this relationship will be facilitated by taking up separately the changes effected: 1, during the 5-minute injection period; 2, 5 to 10 minutes, and 3, 25 to 30 minutes after injection; and 4, the total, summated effect for the entire 35-minute experimental period.

1. *Change in carbon dioxide output and respiratory quotient during the 5-minute injection* (figs. 1 and 2, parts I, continuous line): it will be seen that changes of both are so alike that description of one applies without qualification to the other; and, equally significant, both follow exactly the

same course as previously described for pulmonary ventilation (1940b). All three are maximally increased during the injection of 0.001 mgm. per kilo per minute; and, with minor irregularity probably of experimental origin and evident equally in each (i.e., of the results produced by 0.004 and 0.007 mgm. per kilo per minute, one is either slightly too low or the other too high) the effect is progressively less at each higher rate of injection.

Oxygen consumption during injection (previous report, 1940a) followed a totally different course; being increased approximately the same amount (between 6 and 8 per cent) by all rates of injection during this 5-minute injection-period itself. It therefore seems improbable that carbon dioxide output during this period of adrenalin action is of significance as a measure of the intensity or kind of metabolic process underlying the calorigenic effect of adrenalin.

Blood lactic acid was also affected quite differently (previous report, 1939b), increasing progressively (with minor fluctuations, again of probably experimental origin) from threshold to a maximum with the highest rate of injection. The exactly reciprocal variation of carbon dioxide output can in no way be taken, then, as directly related to the lactic-acid acidosis produced by adrenalin.

In the immediately previous report, also, it was shown that the changes of pulmonary ventilation during injection followed neither metabolic need as expressed by oxygen intake, nor acidosis as represented by blood lactic acid level; but were explainable only as an integrated response to these as modified by the inhibitory effect of a progressively increasing blood pressure. Since there is no means by which this latter could bear immediately upon carbon dioxide production, it must be concluded that in the exactly parallel variation of ventilation and carbon dioxide output it is the former which is determinative; i.e., carbon dioxide output in this phase of adrenalin action (during injection) has no metabolic significance, being to an indeterminate extent merely a passive result of varying degrees of "auspumpung" superimposed upon a probably steady increase in oxidative formation plus displacement by a lactic-acid acidosis.

Obviously (fig. 2) respiratory quotient could be substituted for carbon dioxide output in the above without change of meaning or interpretation. And since evidence on this point was one of the main objectives of the work which has been described in this series of reports it seems justifiable to point out that this conclusion, for which there has been some previous evidence (Erichson, 1926) appears hitherto never to have received such substantial proof as that afforded here.

Carbon dioxide output and respiratory quotient (2) 5 to 10, and (3) 25 to 30 minutes after injection (figs. 1 and 2, parts I, dashed and dotted lines, respectively): there is no need to consider the effects at these intervals after

injection in detail. In general, hypertension with its reciprocally inhibitory effect is eliminated as a decisive influence on pulmonary ventilation so that this (previous report) and, therefore, carbon dioxide output and respiratory quotient must be conditioned during this half-hour recovery period chiefly by metabolic rate and the lactic-acid acidosis.

Metabolic rate 5 to 10 and 25 to 30 minutes after injection, as measured by oxygen consumption, has been shown (1940a) to increase to a maximum following adrenalin administration at the rate of 0.004 mgm. per kilo per minute and thereafter to decline progressively after injection at the two highest rates; carbon dioxide output (fig. 1, dashed and dotted lines) during this period of recovery reflects, qualitatively, these variations of metabolic rate up to injection at the rate of 0.007 mgm. per kilo per minute. It is not to be supposed, however, that even within these rates of administration it is at these times quantitatively related to metabolic rate; displacement by the progressively increasing lactacidemia must contribute to the measured output and following the highest rate of injection becomes predominant; output being then increased in spite of the fact that oxygen consumption is not even appreciably affected (1940a).

Respiratory quotient (fig. 2, dashed and dotted lines) merely reflects these complicated relationships in a manner too intricate to justify or repay attempted analysis; particularly, its rapid elevation during this phase of recovery when injection has exceeded 0.004 mgm. per kilo per minute can have no metabolic significance.

4. *Total, summated changes of carbon dioxide output and respiratory quotient for the entire 35-minute experimental period* (figs. 1 and 2, parts II): it is possible that employment of sufficiently long experimental periods would permit compensation for the disturbances of carbon dioxide output and respiratory quotient that have been shown above to be characteristic of short-period determinations. This evidence indicates, however, that such compensation is not effected within half an hour after injection. Comparison of these curves with those previously given for blood lactic acid (1939b), oxygen consumption (1940a), and pulmonary ventilation (1940b) only emphasizes the conclusion already reached: that carbon dioxide output and, therefore, respiratory quotient following administration of adrenalin are merely unanalyzably complex resultants of the interaction of these three determinants; or, rather, follow more closely than any other the variations of pulmonary ventilation; which, in turn, is determined by the other two.

COMMENT. A study of the result of injecting adrenalin at any one of the effective rates employed in this work would reveal increased blood sugar and lactic acid concentrations, increased oxygen consumption, carbon dioxide output and respiratory quotient, and increased pulmonary ventilation; and the attempt to establish causal relationships between

any or all of these would have to depend on the degree of synchronism of the various changes. This has received considerable attention (for literature and pertinent data see Erichson, 1926) but for reasons not far to seek has been inconclusive. Thus as shown in this work, after injecting 0.004 mgm. per kilo per minute there is increase of all these variables during the 5-minute injection; this increase, for all except respiratory quotient continues at least into the 5–10 minute interval following and then all show parallel rate of decline toward normal. Following any other rate of injection, however, these relationships and correlations will be quite different so that conclusions based on such evidence will necessarily vary from one set of data to another depending on the effective concentration of adrenalin which is established by the method of administration employed. It was a suspicion of this which suggested that in so far as changes of any of these were of common or dissimilar origin the most crucial proof thereof would be their degree of alteration in response to varying concentrations of adrenalin, i.e., their proportionality to dose or stimulus.

It is this effect which has been described in this series of reports of which this is the last. These have shown that on this basis there is no exact correspondence between the calorigenic effect as measured by oxygen consumption and any of these variables. Perhaps this is not surprising in so far as changes of blood sugar and lactic acid are concerned; although if all three, as seems commonly supposed, are effects of uncomplicated direct cellular action it would not have been too surprising to find some similarity in their variation in response to a common stimulus. Unexpectedly, what little resemblance there is unites calorigenic response with mobilization of blood sugar rather than of lactic acid.

On the other hand it was hardly to be expected that calorigenic action, as measured by oxygen consumption, would show quite such complete independence of carbon dioxide output and respiratory quotient. These results more than substantiate such intimations of this as previously obtained and once for all render untenable any attempt to explain qualitatively the effect of adrenalin on metabolism from short-period measurements of the respiratory exchange.

SUMMARY

Data are presented describing carbon dioxide output and respiratory quotient during, and 5 to 10, and 25 to 30 minutes after, intravenous administration of adrenalin for 5 minutes at rates of 0.00025, 0.00050, 0.00100, 0.00200, 0.00400, 0.00700 and 0.01000 mgm. per kilo per minute.

For the 5-minute injection period, maximum increase of both occurs with 0.001 mgm. per kilo per minute, which appears, also, the minimal effective dose. With each of the four higher injection rates, the increase

during this period of injection is progressively less. Such, also, was the effect on pulmonary ventilation, described in the previous report of this series (1940b); and thus is provided perhaps the most probable explanation of the results described here.

After injection, carbon dioxide output and respiratory quotient also seem most adquately explained by pulmonary ventilation, so, again, reference may be made to the description of it (1940b) for explanation.

Most importantly, neither carbon dioxide output nor respiratory quotient vary in proportion to dosage with any similarity to oxygen consumption and would therefore seem very unreliable indices of the qualitative metabolism underlying the calorigenic action of adrenalin.

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SOME FEATURES OF THE EARLY STAGES OF NEUROMUSCULAR TRANSMISSION

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In previous communications from this Laboratory (Rosenblueth and Morison, 1937; Rosenblueth and Luco, 1939) emphasis has been placed upon the complex sequence of variations of neuromuscular transmission when a motor nerve is stimulated at adequate rates. Three successive stages of transmission—indicated by initial high muscular tension, then low and thereafter again high tension—were recognized early during repetitive activation. These stages were followed by a further period of depression (the *4*th stage, fatigue) and by a later recovery (*5*th stage).

An occasional early stage of depression, different in its time course from the *2*nd stage, was mentioned by Rosenblueth and Luco (1939). The present study deals with this additional stage, the conditions for its regular appearance, its properties and its mechanism of production. At high-frequency stimulation this stage is later than the *2*nd. Indeed, it appears to take place during the course of the *3*rd stage (fig. 2). In order not to modify seriously the original nomenclature of the several stages the new phase will be spoken of as stage *3b*. The complete sequence of events includes, therefore, the stages *1, 2, 3a, 3b, 3c, 4* and *5*; the three successive periods of depression, during which transmission fails to occur at many of the junctions, are *2, 3b* and *4*.

METHOD. The muscle studied was mainly the soleus of the cat. Occasionally the gastrocnemius or quadriceps was observed, with only slight quantitative differences in the results. The cats were anesthetized with dial (Ciba, 0.75 cc. per kgm. intraperitoneally). The tendon of the muscle was freed and attached to a tension myograph. The muscles pulled against strong rubber bands. Upward excursions in the records denote contraction. The leg was fixed by drills inserted into the tibia or femur.

Stimulating shielded silver-wire electrodes were placed on the motor nerve, cut centrally. The stimuli were condenser discharges through a thyratron, controlled in rate by a frequency-beat oscillator. The intensity was maximal. The time constant of the discharges was approximately 0.2 msec.

The nerve action potentials were recorded sometimes from a pair of electrodes placed on an intact region of the nerve, peripheral to the stimulating electrodes. In some experiments the electric responses of the crushed peroneal nerve were led monophasically from the region near the head of fibula, the stimulating electrodes being, as usual, high in the thigh. The action potentials of the muscles were recorded diphasically from two silver or platinum needles inserted into the tendon and into muscle fibers, respectively. Capacity-coupled amplifiers and a cathode-ray oscillograph were used for the records.

Atropine and prostigmin (Roche) were injected intravenously. Acetylcholine and KCl solutions were injected into the central end of the tied inferior mesenteric artery; this insured a prompt delivery to the recording muscles and permitted the use of smaller amounts of the substances than necessary for intravenous injections.

RESULTS. A. *The stage 3b.* The existence of a secondary stage of depression and the clear temporal separation of this stage from the initial similarly depressed stage *2* were readily demonstrated by the two following experimental procedures. In some animals records were taken of the responses to a series of periods of repetitive stimulation with progressively higher or progressively lower frequencies. Figure 1 illustrates a typical instance. The popliteal nerve was stimulated for 15 sec. at the frequencies indicated, rest periods of 2 min. being allowed between successive stimulations. The existence of two separate stages of depression is clearly seen in the responses to 400 and 500 per sec. As will be shown later (fig. 4), the fall of tension which takes place at 200 and 300 per sec. is not due to fatigue, for if the stimulus is prolonged the tension rises within a few seconds or minutes.

The two depressions, *2* and *3b*, become even more prominent when a high frequency is applied repeatedly for short periods (10 to 15 sec.) with intervening similar short periods of rest. Figure 2 shows a typical record. It is apparent that the response to the first period of stimulation contained exclusively stages *1*, *2* and *3* (now designated *3a*). As the stimuli were repeated *2* became briefer and the bottom of its trough rose while *3b* started earlier and lasted progressively less time.

Repetition of stimuli leads to fatigue. The records in figure 2 may be explained on this basis. As fatigue progresses both *2* and *3b* become briefer, hence the increasing prematurity of their appearance; hence, also, the greater prominence of *3a* and *3c* (see p. 214). That repetition of stimuli within brief periods has also a favorable effect on the appearance of both *2* and *3b* is shown in figure 3. The experiment illustrated therein involved a combination of the two procedures reported above. The stimuli were repeated at regular short intervals and their frequency was first increased and then decreased. A comparison of the responses to 300 and

200 per sec. at the beginning and the end of the series (A and B, J and L-respectively) shows that the repeated stimulation has favored the occurrence of *2* and *3b* with relatively low frequencies. It may be inferred that the post-tetanic condition of the muscles is conducive to the appearance of the early depressions.

That the fall of tension which takes place early during stimulation at frequencies of 200 and 300 per sec. is not due to the development of

Fig. 1   Fig. 2

Fig. 1. Appearance of stage *3b* and later of stage *2* as the frequency of stimulation is increased. Records from soleus. The popliteal nerve was stimulated for 15 sec. every 2 min. Frequencies of stimulation: A, 100; B, 200; C, 300; D, 400; and E, 500 per sec., respectively. Time signal: 5-sec. intervals.

Fig. 2. Effects of repetition of the stimuli upon the stages *2* and *3b*. Soleus. Frequency of indirect stimulation: 500 per sec. The record is continuous, the stimuli being applied repeatedly for 10 sec., with rest periods of 10 sec.
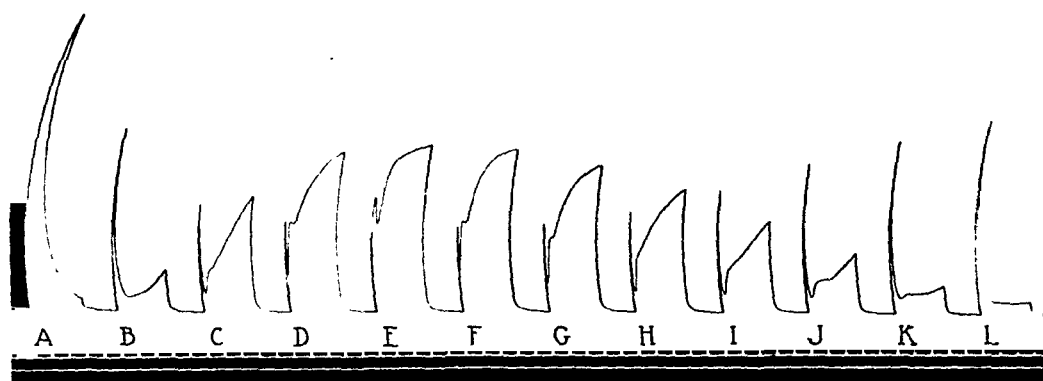
Fig. 3. Post-tetanic influence on the occurrence of *2* and *3b*. Soleus. Continuous record. Stimuli applied for 15 sec. with rest periods of 10 sec. Frequencies: A, 200; B, 300; C, 400; D, 500; E, 600; F, 500; G, 450; H, 400; I, 350; J, 300; K, 250; and L, 200 per sec.

the *4*th stage, but to the beginning of stage *3b*, is shown by the subsequent rise which occurs if the stimuli be prolonged over a period of 1 to 5 min. (fig. 4). With slower frequencies (e.g., 60 per sec.) the *4*th stage does follow the *1*st with no intervening *2* or *3* (Rosenblueth and Luco, 1939).

The occurrence of the several stages of neuromuscular transmission with different frequencies of stimulation may be summarized as follows. With low frequencies of indirect stimulation, e.g., 30 per sec. or less, transmis-
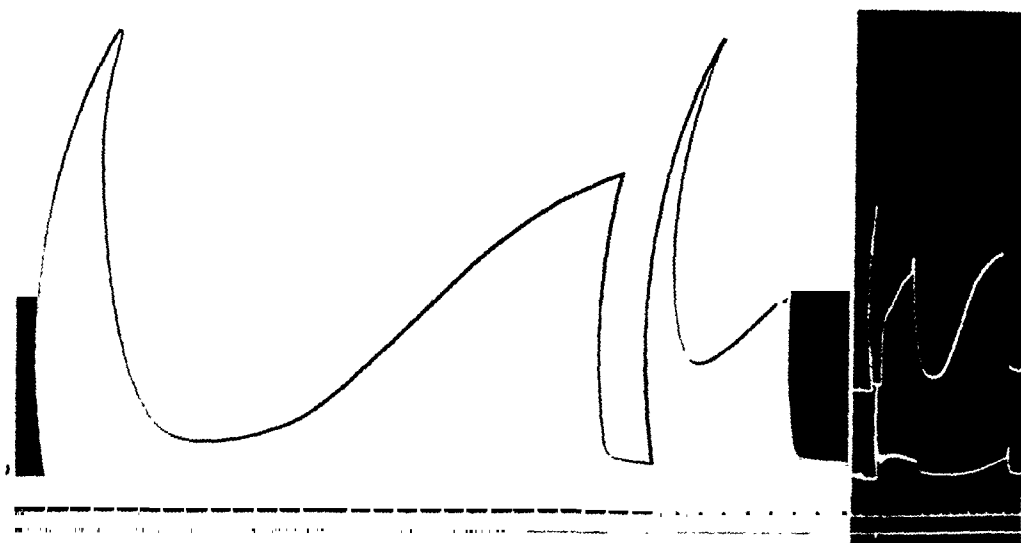
Fig. 4   Fig. 5

Fig. 4. Stage *3b* with a frequency of indirect stimulation of 200 per sec. Soleus. Time signal: 5 sec. After stimulation for 2 min. a rest period of 10 sec. was given. Re-application of the stimuli reveals the influence of fatigue.

Fig. 5. Stages *1, 2, 3a, 3b* and *3c* elicited with a frequency of 30 per sec. after prostigmin. Atropine: 1 mgm. per kgm. Records from the two gastrocnemii. The left popliteal nerve (lower record) had been stimulated at 60 per sec. for 40 min. This stimulus was stopped and prostigmin (0.5 mgm.) was injected. Two min. later a frequency of 30 per sec. was applied to both sides (at the first lower signal). Time signal: 1 min. The drum was faster at the beginning of the response, to spread out the prompt and brief stage *2*.

TABLE 1

Changes in spike magnitude and in conduction velocity of the A fibers of a circulated peroneal nerve when stimulated for 15-sec. periods at different frequencies

The spike magnitude is expressed as per cent of the response of the rested nerve stimulated at a low frequency. The conduction velocity, expressed as m. per sec., refers to the fastest fibers in the nerve. The last four determinations at 500 per sec. were made successively with 10-sec. rest intervals between the periods of stimulation.

FRE-QUENCY PER SEC.	SPIKE MAGNITUDE					CONDUCTION VELOCITY			
	First spike	1 sec. later	5 sec.	10 sec.	15 sec.	1 sec.	5 sec.	10 sec.	15 sec.
100	100	105	103	105	100	97	100	100	98
200	100	110	105	105	105	100	98	97	97
300	100	107	103	103	100	97	95	95	93
400	100	104	103	99	97	93	90	90	89
500	100	103	99	99	95	90	88	85	82
600	100	110	103	101	100	83	78	75	74
1,000	100	86	62	43	41				
500	100	108	104	102	100	90	90	85	84
500	100	108	104	100	100	89	84	82	82
500	100	102	100	100		85	84	82	
500	100	108	102	100		87	82	80	

sion remains in the *1*st stage, even when the stimuli are applied for several hours (Rosenblueth and Luco, 1939). With frequencies between 50 and
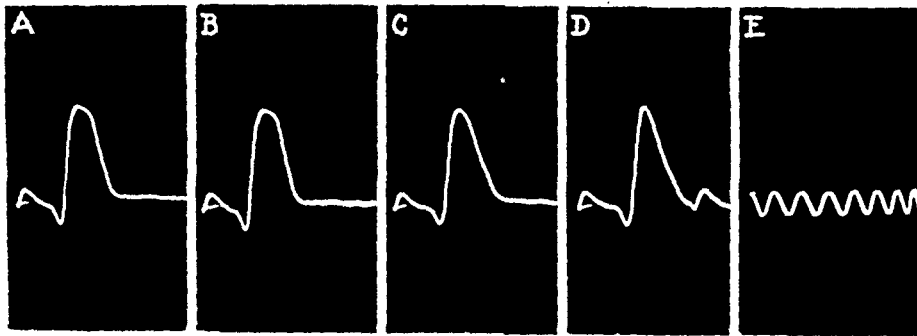
Fig. 6. Responses of the A fibers of a circulated peroneal nerve to high frequency stimulation. Sciatic cut centrally. Stimulating electrodes immediately below the cut. Recording electrodes near fibula; nerve crushed between them. Conduction distance 7 cm. The stimuli were made to trip the sweep of the cathode-ray oscillograph; the records begin, therefore, with a stimulus artifact.

A. Beginning of stimulation at 100 per sec. B. 15 sec. later. C. After 15 sec. stimulation at 300 per sec. D. After 15 sec. stimulation at 500 per sec. E. Calibration of sweep: 1,000 cycles.
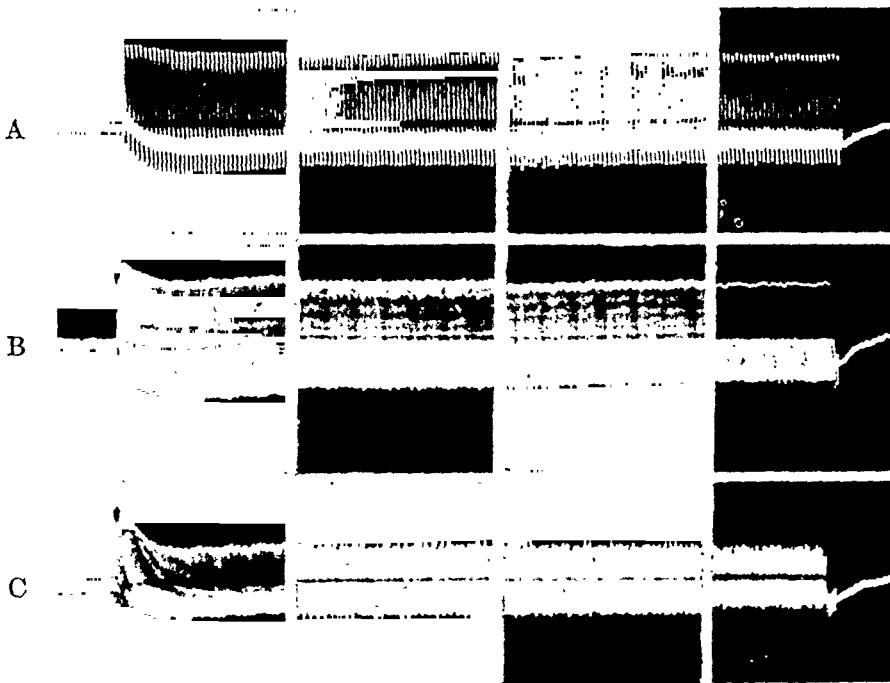
Fig. 7. As in figure 6, but continuous film to emphasize changes in spike-magnitude. The stimuli were applied for 15 sec. and pictures were taken at the beginning, 5 and 10 sec. later, and at the end of the period of stimulation.

A. Frequency, 400 per sec. B. Frequency, 800 per sec. C. Frequency, 1,000 per sec.

100 per sec. the *1*st stage is followed by the *4*th, and later by the *5*th. Between 150 and 300 per sec. the sequence is $1 \rightarrow 3b \rightarrow 3c \rightarrow 4 \rightarrow 5$.

With frequencies higher than 300 per sec. *1* is followed by *2*, then by *3a*, *3b*, and the sequence is thereafter as just given.

B. *The nerve action potentials.* Since there is little information available on the behavior of circulated mammalian nerves stimulated for relatively long periods with high frequencies, as was done in these experiments, the question arose whether the striking changes in tension illustrated above (figs. 1 to 4) could be due to corresponding changes in the number of nerve impulses delivered to the muscle per unit time. Accordingly the spike potentials of the A fibers of circulated peroneal nerves were recorded when stimulated as in the previous experiments.

Figures 6 and 7 illustrate typical results. For a more detailed description of the behavior of the nerves all the results of one experiment are sum-
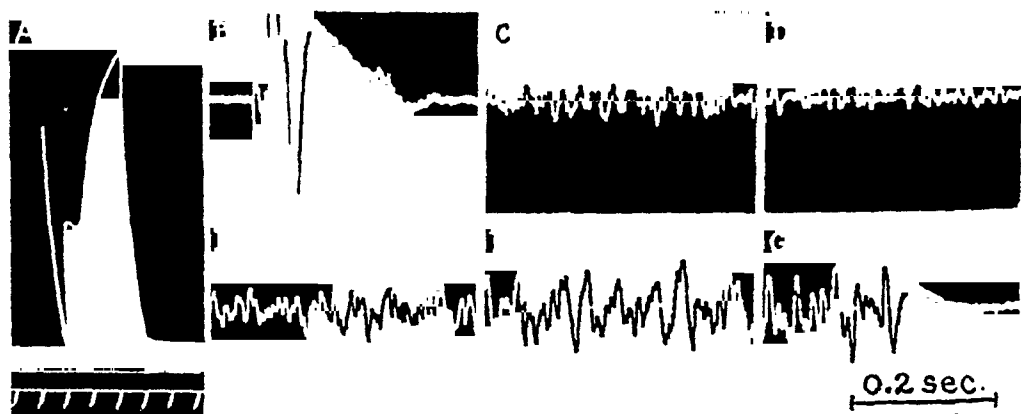
Fig. 8. Mechanical and electrical responses of a soleus muscle to high frequency (600 per sec.) stimulation of the popliteal nerve.

A. Mechanogram. Time signal, 5 sec. B to G. Electrograms. B, beginning of stimulation; the first spike potential went off the record. C to F at approximately 3-sec. intervals. G, end of stimulation.

marized numerically in table 1. With frequencies up to 600 per sec., although the conduction velocity is slowed, no significant change of spike magnitude of the α fibers takes place. Alternation was never apparent with frequencies up to 800 per sec., and was obvious at 1,000 per sec. only after a few seconds of stimulation. It is clear that the minor changes in the nerves are totally unrelated to the striking variations of neuromuscular transmission which correspond to the different stages.

C. *The muscle action potentials.* The electric responses of the muscles varied with the different frequencies of stimulation employed. It is not necessary for present purposes to give a detailed description of all the changes encountered. The following general statements may be made. At high frequencies of stimulation (300 or more) the muscle electrograms did not follow the rate of stimulation. Figure 8 is typical of the irregular

records obtained in all instances. Such irregularities are clearly indicative of asynchronism and alternation.

Notwithstanding the complex electromyograms recorded it was apparent (fig. 8) that the tension and the electric responses varied in a parallel manner. This parallelism warrants the conclusion that the changes of tension recorded indicate mainly or exclusively variations of contraction, not of contracture.
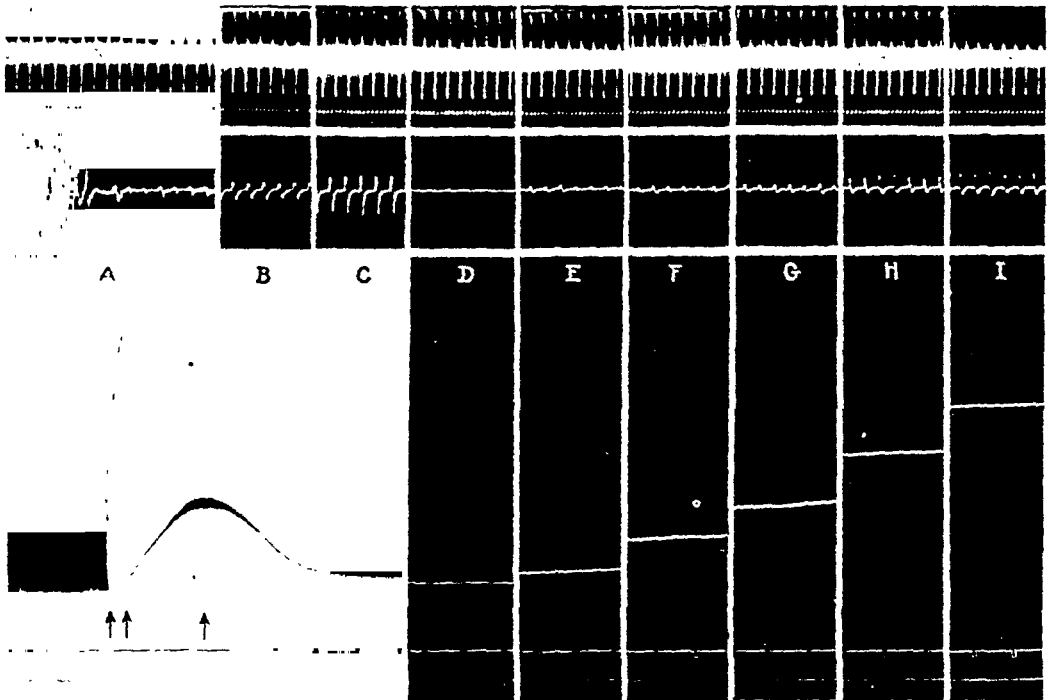
Fig. 9. Responses of nerve and muscle to a prolonged period of stimulation at 30 per sec. after prostigmin (0.5 mgm.). Upper records: nerve electrogram recorded diphasically from the stimulated popliteal nerve. Time: 10 msec. Middle records: muscle electrogram recorded diphasically from needles in the tendon and belly of gastrocnemius. Lower records: mechanogram. Time: 5 sec.
A. Beginning of stimulation. B and C. 3 and 12 sec. later, respectively (arrows). D to I. 2, 5, 6, 7, 10 and 14 min. later, respectively.

D. *Prostigmin.* Injections of prostigmin favor the appearance of stages *2* and *3b*—i.e., these stages occur with slower rates of stimulation than are necessary for their production in the normal systems. The results were most clear and striking when a slow frequency (30 to 60 per sec.) of stimulation was applied after atropine and prostigmin to a nerve which had not been stimulated previously. Figure 5 (upper record) illustrates a typical instance. Figure 9 shows that the nerve action potentials remain full-sized throughout the period of stimulation and that the changes of tension are due to increasing or decreasing numbers of muscle fibers sharing

in the response, as indicated by the variations in the muscle action potentials. When muscles had been stimulated for some minutes before the injections of prostigmin the stages in question did not appear as clearly as in fresh preparations (fig. 5, lower record).

When, after prostigmin, a high frequency of stimulation (100 per sec. or more) is applied to the motor nerve for a brief period (1 or 2 sec.)
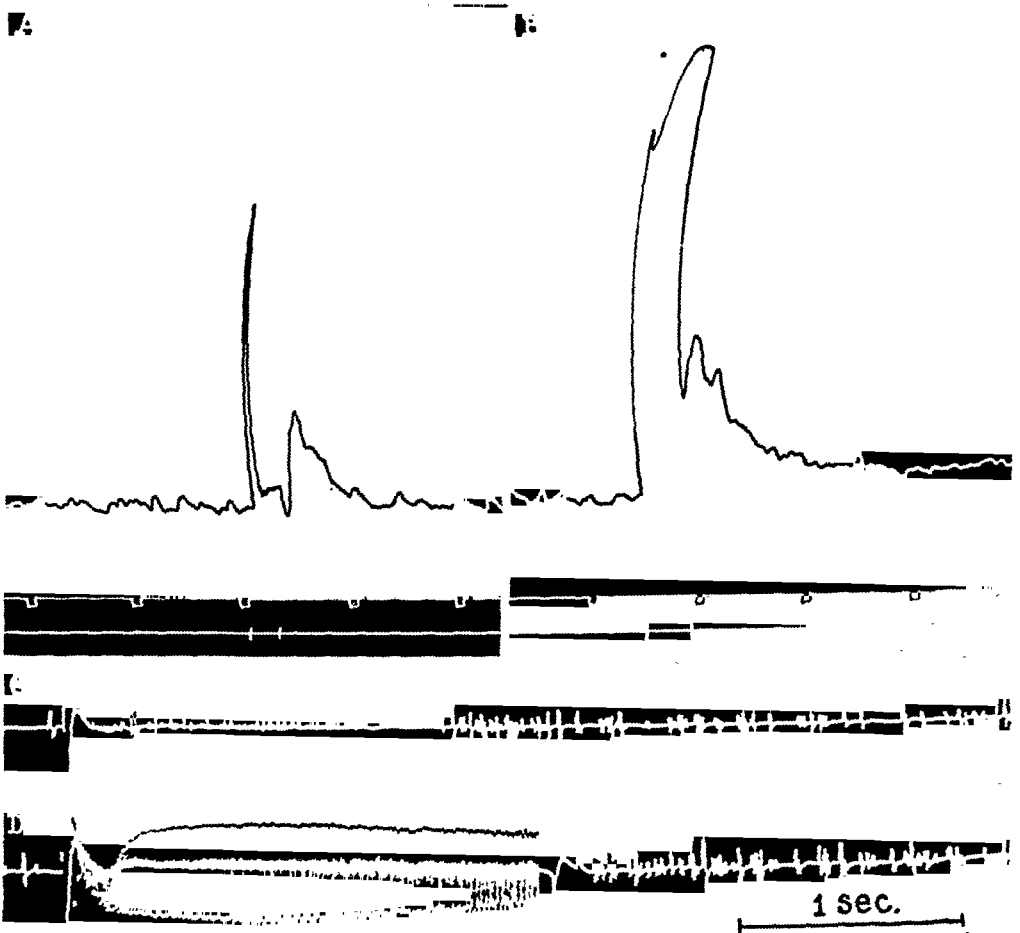
Fig. 10. Delayed contractions following repetitive stimulation after prostigmin. Soleus. Upper records: mechanograms; time signal: 5 sec. A, stimulation of popliteal nerve at 200 per sec. during period marked by lower signal. B, similar stimulation, but at 100 per sec. Lower records: muscle electrograms, C and D corresponding to the mechanograms A and B, respectively.

a delayed increase of tension takes place after stimulation has ceased (Rosenblueth and Morison, 1937). This phenomenon was frequently encountered in the present observations. The electromyograms (fig. 10) exhibit the presence of action potentials during this late response. The rise of tension is due, therefore, to a contraction, not a contracture.

E. *Acetylcholine.* Intra-arterial injections of acetylcholine were made

at different times during a period of stimulation at high frequency, in order to test the effects of these injections on the several stages of transmission. The results may be summarized as follows. As pointed out by Rosenblueth and Morison (1937), acetylcholine produces a fall of tension when injected during stages *1* or *3a*. This fall was then interpreted as the appearance of stage *2*. This interpretation is invalidated by the present observations. Acetylcholine had only a slight and inconsistent effect on stage *2* (fig. 11A). On the other hand, it caused a marked accentuation and prolongation of stage *3b* (fig. 11A and B). The fall of tension which
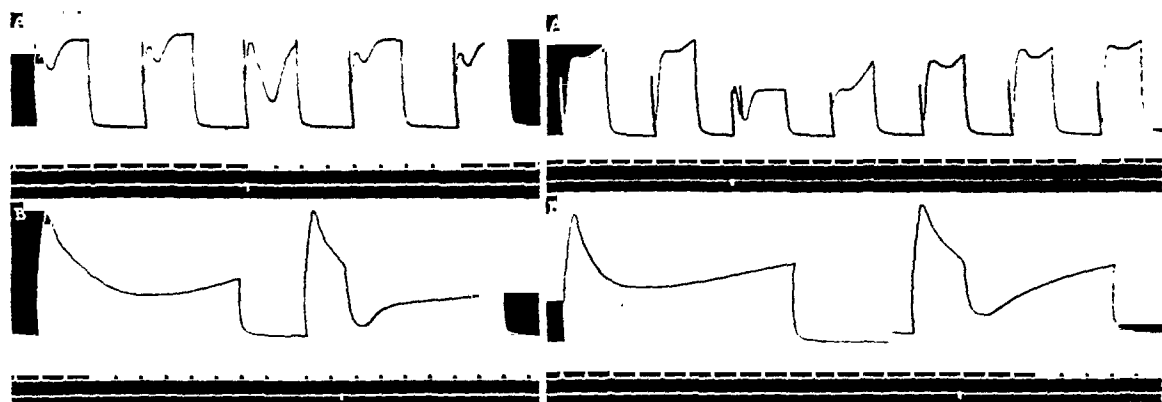
Fig. 11   Fig. 12

Fig. 11. Influence of acetylcholine on stages *2* and *3b*.

A. Plantaris. The popliteal nerve was stimulated at the rate of 600 per sec. for 10 sec. with 10-sec. rest intervals. Several such stimuli had been applied before the record began, so that a steady state of the responses had been attained. At lower signal (i.e., at the start of a period of stimulation) acetylcholine (1 mgm.) was injected into the inferior mesenteric artery. Time signal: 5 sec.

B. Soleus. The first response shows the result of stimulation at 200 per sec. A similar response is markedly depressed by the injection of 0.5 mgm. acetylcholine at the lower signal. Time: 5 sec.

Fig. 12. Influence of potassium on stages *2* and *3b*. Records and procedure as in figure 11, except that KCl (20 mgm. in A, 25 mgm. in B) was injected instead of acetylcholine.

acetylcholine produces when delivered during stage *1* is therefore probably due to the development of stage *3b*.

F. *Potassium.* The results of intra-arterial injections of KCl were qualitatively similar to those of acetylcholine described above. Small doses (5 to 15 mgm.) had a brief action, while larger doses (20 to 50 mgm.) had prolonged effects. Figure 12 illustrates typical observations.

G. *Post-tetanic effects.* It is well known that the responses of muscles to different stimulating agents and procedures undergo characteristic variations when tested shortly after a period of tetanization. In the course of this study several such changes were encountered, which will be described in this section.

Rosenblueth and Morison (1937) reported that the muscular responses to single nerve volleys could become repetitive during the post-tetanic period. This report was called erroneous by Brown and Euler (1938). Feng, Li and Ting (1939a), however, gave clear electrical evidence of the repetitive nature of the post-tetanic responses of soleus. We were able to confirm fully these observations.

The responses of denervated muscles to acetylcholine were found by Rosenblueth and Luco (1937) increased after a period of direct tetanization. In the present experiments the responses to both acetylcholine and potassium were greater after short periods (10 to 30 sec.) of indirect activation. Indeed, doses which were quite subliminal before the tetanus yielded marked effects during the post-tetanic stage. Such responses were con-
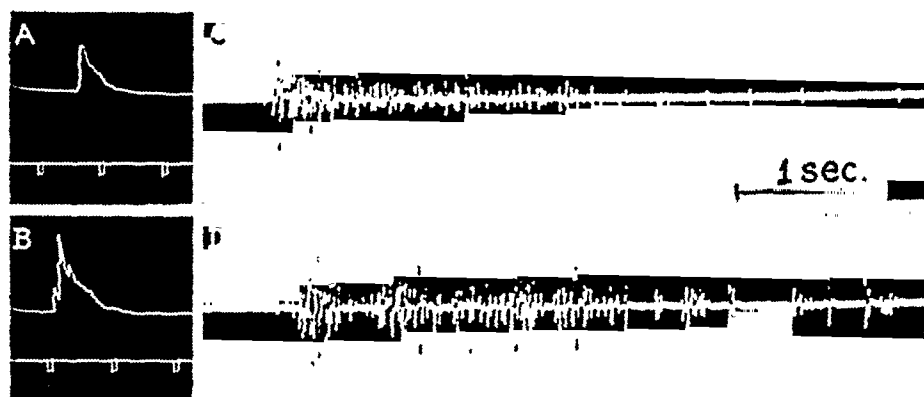
Fig. 13. Mechanical (A and B) and electrical (C and D) responses of a soleus muscle to intra-arterial injections of acetylcholine (1 mgm., A and C) and KCl (50 mgm., B and D). These injections were made shortly after several periods of tetanic stimulation. The same doses injected before tetanic stimulation gave no responses, mechanical or electrical.

tractions, as shown by the presence of spike-potentials in the electrograms (fig. 13).

DISCUSSION. Changes of tension early during repetitive indirect stimulation at high frequency had been observed by Wedensky (1886) and Hofmann (1902). A recognition that such changes of tension imply different stages of neuromuscular transmission was, however, not made at that time.

The records in figures 2 and 4 indicate that stages 2 and 3b are not phenomena of fatigue or exhaustion. If such were the case repetition of the periods of stimulation at short intervals should lead to increasing fatigue, and hence to a deeper and more prolonged fall of tension at those stages. The opposite effect is observed: stages 2 and 3b become briefer and less prominent as fatigue develops. The paradoxical increase of response dur-

ing fatigue is explained by the assumption that stages *2* and *3b* are not caused by deficiency or exhaustion, but are due to the superabundance of a depressive agent or condition.

Repetition of the periods of stimulation does result, in certain observations, in an increase of stages *2* and *3b* (figs. 2 and 3). It may be inferred that a previous period of tetanization conditions the succeeding response in two opposite ways. It tends to decrease the response (both the + and the − stages) because of fatigue. But it also tends to increase these responses because of the development of another factor, unknown at present. This factor is assumed to be responsible for the changes described in section G under the heading "post-tetanic effects." It will be discussed further below.

Before considering the possible mechanisms involved in the appearance of the several stages it is important to emphasize some of the negative aspects of the problem. The records of the nerve spike-potentials (figs. 6, 7 and 9, table 1) render unlikely the view that changes in the magnitude of the nerve impulses delivered to the muscle can be responsible for the sequence of the stages. It is true that these spike-potentials are measured from the axons in the nerve trunks, not at the fine terminal filaments or nerve endings. But there is no reason to believe that such fine nerve terminals differ significantly in their properties from the regions of the axons tested. Indeed, the sequence of the stages takes place after prostigmin at such low frequencies (figs. 5 and 9) as to render quite improbable the view that the nerve impulses would vary significantly during the period of stimulation.

The electromyograms (figs. 8 and 9) indicate that the changes of tension are due to variations of contraction, not to the presence or absence of contractures. Although systematic data on the ability of the conducting system of muscles to follow different frequencies are not available, the incidental observations made in this and previous studies would eliminate changes of muscular conduction as the source of the sequence of the stages. At slow frequencies (fig. 9) the muscle should be capable of following the stimuli throughout and although at high frequencies the muscle cannot follow the rate applied to the nerve, typical action potentials are elicited at a slower rate (fig. 8).

It may be inferred that the sequence of stages denotes fundamentally changes of neuromuscular transmission, as opposed to possible changes in the conducting systems of either nerve or muscle.

As far as is known at present, there is no significant difference in the process of transmission during stages *1*, *3a* and *3c*. The stimulus delivered by the nerve to the muscle is adequate. There is no alternation in the muscular responses at low frequencies, and, if the rate of the nerve impulses

is too high for the muscle to follow, alternation is grossly similar in these three stages. The action of acetylcholine, potassium and prostigmin is likewise similar.

The stages of depression or lack of transmission, *2*, *3b* and *4*, on the other hand, differ significantly from each other, as follows. With respect to frequency of stimulation, stage *4* occurs at lower frequencies than the other two; a frequency of 50 per sec. is adequate for its appearance in gastrocnemius or soleus (Rosenblueth and Luco, 1939). Stage *3b* takes place with frequencies of 200 to 300 per sec. (figs. 1, 3 and 4), while stage *2* requires frequencies of 400 or more (figs. 1, 2 and 3). Acetylcholine and potassium have only a slight effect on stage *2* (figs. 11 and 12); they markedly accentuate stage *3b* (i.e., the tension falls lower; figs. 11 and 12); they improve transmission during stage *4* (i.e., the tension rises; Rosenblueth and Morison, 1937).

When a single early stage of depression is present in the response to stimulation under given experimental conditions, it is sometimes difficult to decide whether that stage is *2* or *3b*. If the action of acetylcholine or potassium can be tested the doubt may disappear. It is likely, however, that in certain conditions—e.g., rapid stimulation after prostigmin— stage *2* is long-lasting and stage *3b* begins sooner than usual, so that the two stages merge and stage *3a* is totally absent. Such a sequence can only be inferred.

Rosenblueth and Morison (1937) attributed stage *2* to an excess of acetylcholine causing the well-known paralytic action. This view is not supported by the present data. For, if stage *2* were due to an excess of acetylcholine, injections of the substance should result in further depression, yet they do not (fig. 11). On the other hand, stage *3b* appears to be produced by precisely that mechanism (fig. 11). The excess of acetylcholine could be absolute, because of accumulation during repetitive stimulation, or only relative, because the muscle presents to the transmitter a lower paralytic threshold at that time. That high frequency of stimulation will render the muscle more excitable to acetylcholine is shown in figure 13. This increased excitability after high-frequency activation is in contrast with the decreased excitability which is apparent after low-frequency tetanization (Rosenblueth, Lissák and Lanari, 1939). The complexity of the after-effects of tetanic stimulation is thus emphasized.

If stage *2* is not due to an excess of acetylcholine, what may be its source? This question is at present unanswerable. Clearly some depressive factor different from acetylcholine is at play. Indeed, since acetylcholine, which deeply modifies the responses of the muscle at all other stages, has a minor effect during stage *2*, it is necessary to assume further that the development of this factor is inversely related to the concentration of acetylcholine. Boyd, Brosnan and Maaske (1938) have described an early inhibitory stage

apparent in neuromuscular systems treated with magnesium. Although the data are not sufficient for a decisive conclusion, it is possible that this inhibition may differ from stage 2, since the authors conclude that their depression is more marked at lower than at higher rates of stimulation, whereas 2 is in direct relation with the frequency of stimulation (figs. 1 and 3).

Feng, Li and Ting (1939a) report that the repetitive response of a muscle to a single nerve volley during the post-tetanic period is followed by an inhibitory after-effect. This inhibition is decreased by eserine. It is furthermore independent of the frequency of stimulation (Feng, Li and Ting, 1939b), the first volley producing as much after-depression as a series of volleys. Since stage 2 is more prominent after eserine and since it is undoubtedly a function of the frequency of stimulation, it may be inferred that stage 2 and Feng, Li and Ting's inhibition are due to different processes.

It may be concluded that the processes which may lead to cessation of neuromuscular transmission are numerous and complex. The explanation suggested by Rosenblueth and Morison (1937) for stage 4, that it is due to an exhaustion of the production of acetylcholine, fits all the data (cf. Rosenblueth, Lissák and Lanari, 1939). Stage 3b may well be due to an excess of acetylcholine; no data contradict this view. At least two more conditions of depression should be recognized: stage 2 and Feng, Li and Ting's inhibition. More data are necessary before deciding upon a reasonable explanation for these two types of depression which will be more than speculative.

The increment of responses to single nerve volleys during the post-tetanic period was attributed by Rosenblueth and Morison (1937) to a mobilization of potassium ions in the muscle. A similar explanation has been adopted by Brown and Euler (1938) and by Feng, Lee, Meng and Wang (1938). In accord with this view Lee (1939) reports that there is in denervated muscle an inverse correlation between the potassium contents and the excitability to acetylcholine. Before applying Lee's evidence to the problem under discussion it would be necessary, however, to learn whether the responses of the muscles he tested were contractions or contractures.

The data (figs. 11, 12 and 13) support the close interrelation between acetylcholine and potassium in neuromuscular transmission previously suggested by many observations. The nature of this interrelation is, however, at present quite obscure. It is likely that light on this problem might elucidate many of the difficulties now encountered.

SUMMARY

The responses of striated muscles to stimulation of their motor nerves were studied in cats. With a high frequency of stimulation the tension undergoes the following typical sequence of ups and downs. The initial

rise (stage *1*) is promptly followed by a fall (stage *2*); a further rise (*3a*) is again followed by another fall (*3b*); a new rise (*3c*) marks the end of the "early" stages; these are followed by the "late" stages, fatigue (*4*) and a delayed rise (*5*).

When tests are made with increasing frequency of stimulation stage *3b* occurs with a lower rate than is necessary for the appearance of stage *2* (figs. 1, 3 and 4). Repetition of a high frequency results in the earlier development of the stages of depression *2* and *3b* (figs. 2 and 3).

The depressions are not due to corresponding decreases in the nerve impulses delivered to the muscles (figs. 6, 7 and 9; table 1).

The changes in tension are due to variations of contraction, not of contracture (figs. 8 and 9).

After prostigmin the early stages may occur with relatively slow frequencies (figs. 5 and 9). A brief period of stimulation at a rapid rate is usually followed by a delayed contraction (fig. 10).

Acetylcholine and potassium have only a slight effect on stage *2*. They accentuate the depression during stage *3b* (figs. 11 and 12).

Tetanic stimulation augments the muscular responses to acetylcholine and potassium (fig. 13).

The discussion of the data leads to the following inferences. The changes of tension are due to presence or absence of transmission at some of the neuromuscular junctions—i.e., the stages denote changes of transmission (p. 215). No explanation is available for stage *2* (p. 216). Stage *3b* can be explained by the assumption of a paralytic effect of an excessive concentration of acetylcholine (p. 216). The increased responsivity of muscle to nerve impulses, to acetylcholine, and to potassium after a period of tetanization may be due to a mobilization of potassium during the tetanus, but direct evidence is lacking of such mobilization and its mechanism (p. 217).

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SOME CONDITIONS AFFECTING THE LATE STAGES OF NEUROMUSCULAR TRANSMISSION

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When a motor nerve is continuously stimulated at the rate, e.g., of 60 shocks per second the muscular response becomes minimal as time passes. This stage of "fatigue" is followed gradually by a remarkable recovery of the ability of the muscle to contract (Luco and Rosenblueth, 1939). Because of earlier variations in the behavior of muscle stimulated indirectly these two stages have been numbered, respectively, *4* and *5*. In an assay of the acetylcholine (a-ch) content of motor nerves which had been stimulated until the fatigue of stage *4* or the full recovery of stage *5* had been reached, Rosenblueth, Lissák and Lanari (1939) found that the the concentration of a-ch was low in stage *4* and that it gradually increased with the degree of recovery in stage *5*. According to the theory of chemical transmission at neuromuscular junctions, stage *4* results from *transmission fatigue*, i.e., the output of a-ch is not adequate to excite contraction in many of the muscle fibers; and in stage *5* the muscular performance is better because the nerves, richer in a-ch, are liberating more of it at their terminals. Support for this interpretation of stages *4* and *5*, or lack of it, is important for acceptance or rejection of the chemical theory. Further pertinent evidence can be obtained by testing the effects of prostigmin and curare and by registering the influence of tetanic stimulation in the two stages.

METHODS. The method employed was essentially that described by Luco and Rosenblueth (1939). In cats under dial anesthesia the Achilles tendons, which were attached to two similar levers, pulled downward against heavy rubber bands. Usually all three muscles—gastrocnemius-plantaris-soleus—were left attached to the tendon, but occasionally the soleus was used alone. For rigid support the legs were fixed by drills driven into the tibiae and firmly clamped to reinforced uprights. Shielded electrodes applied to the popliteal nerves, after severance of the sciatic as near to its origin as convenient, carried the condenser discharges (regulated by a thyratron) which served for stimulation. Shocks of such intensity as to produce maximal single contractions were invariably used.

RESULTS. *The action of prostigmin in stages 4 and 5.* Because pro-

stigmin protects a-ch from rapid destruction by cholinesterase it might be expected to improve muscular contraction in stage 4 by favoring greater effectiveness of the meager output of a-ch from the nerve terminals at that time. In stage 5 it might have either favorable or unfavorable effects, dependent on whether the preservation of a-ch increased the concentration of that agent strictly within the stimulatory range or raised the concentration above the paralytic threshold. Furthermore, since stage 5 develops concomitantly with an increase of a-ch in the nerve, prostigmin might be expected to affect the rate of its development.

In preparation for the tests the animals were secured against the harmful action of prostigmin by an intravenous injection of atropine (1 mgm. per kgm.) just before stimulation was started. The stimuli were repeated at the rate of 60 per second. The popliteal on one side was stimulated until stage 5 was first evident, whereupon stimulation was started on the other side. When on this second side stage 4 was approached or actually reached, prostigmin was injected into the jugular vein. Figure 1 is an illustrative instance of the difference of the effects of a small dose on the two responses —mainly a depressive action in stage 5 and a purely augmentive action when stage 4 was about to be entered. It is noteworthy that the further the progress into a fully developed stage 5 the more direct is the depression induced by prostigmin (cf. fig. 1). And when stage 5 is more marked on one side than on the other, as indicated by a greater degree of tetanic contraction, the effect of prostigmin in causing relaxation is greater on the more contracted side.

Tests with single shocks revealed that the potentiative action of a small dose of prostigmin (about 0.1 mgm. per kgm.) will last at least 90 minutes; and other tests, e.g., on the appearance of stages 2 and 3b (cf. Rosenblueth and Cannon, 1940), indicated that its influence may persist for even longer periods. It was reasonable, therefore, to look for an effect of prostigmin on the time required for the first sign of stage 5 after the stimulation started, and also on the rate of development of stage 5 after it was started. The procedure used to secure evidence on these points was to stimulate the popliteal on one side until stage 5 began to be manifest, and then inject prostigmin (usually 0.5 mgm.) and initiate stimulation of the other popliteal. In six experiments stage 5 invariably developed sooner under the influence of prostigmin than in its absence. The period was shorter by intervals varying from 15 to 60 minutes (see table 1). Usually the time required for the first appearance of stage 5 after the start of stimulation (at 60 shocks per sec.) lies between 90 and 120 minutes. Not only does prostigmin advance the beginning of stage 5, it also accelerates its development. For example, the degree of shortening of the muscles in stage 5 that had been reached just before the first injection of prostigmin at 12:08 in figure 1 had required 43 minutes from the start of that stage.
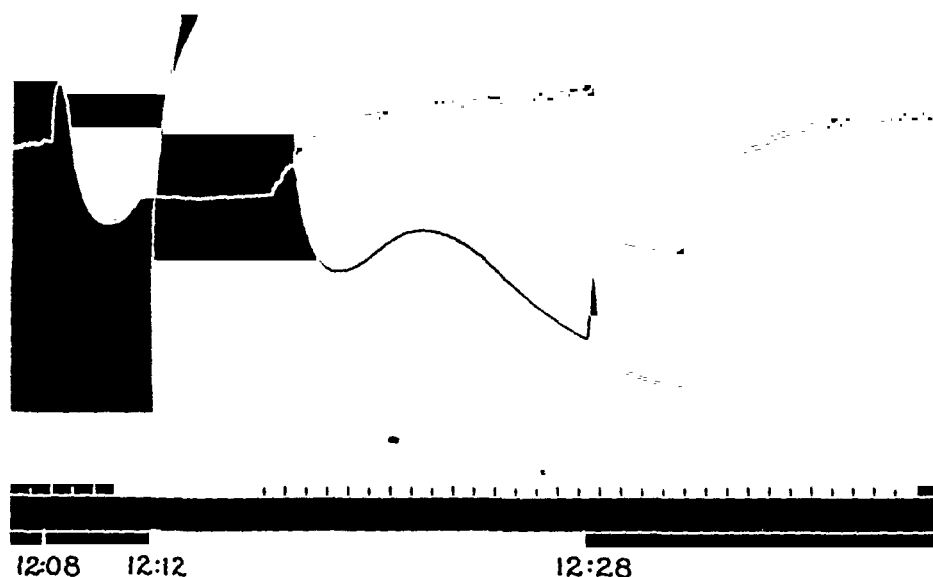
Fig. 1. The effects of prostigmin in stage 5 (upper record) and near the beginning of stage 4 (lower record). At 12:08 prostigmin (0.25 mgm.) was injected into the jugular vein with no obvious effect on the resting muscle (lower record before stimulation). After 12:12 the drum was turned rapidly in order to bring out stage 2 when the stimulation of the resting side was started; stages 3a, 3b and 3c follow. At 12:28 prostigmin (0.25 mgm.) caused a pure contraction on the side approaching stage 4, and on the side in stage 5 almost a pure relaxation. Time, 1-minute intervals.

TABLE 1

Time of first appearance of stage 5 after the start of stimulation

In each experiment are compared bilaterally symmetrical muscles in the same animal.

WITHOUT PROSTIGMIN	AFTER PROSTIGMIN	SHORTENING OF THE PERIOD
min.	*min.*	*min.*
85	65	20
105	45	60
118	65	53
75	60	15
105	75	30
135	120	15

After the two injections of prostigmin (0.25 mgm. each) the same degree of shortening in stage 5 was reached by the muscles of the other side in 22 minutes. This more rapid increase of tension in the recovery phase after

fatigue, as the stimulation is continued under prostigmin, is a quite typical phenomenon.

The relative effects of curare in stages 4 and 5 and at the fresh neuromuscular junction. Curare reduces the muscular response to uniform maximal nerve impulses or to uniform injections of a-ch (Brown, Dale and Feldberg, 1936; Rosenblueth and Luco, 1937). By increasing the amount injected the response is decreased. In short, curare acts as if it raised the threshold to a-ch. Since there is evidence that nerve impulses discharge different amounts of a-ch in stages 4 and 5 and when there has been no previous
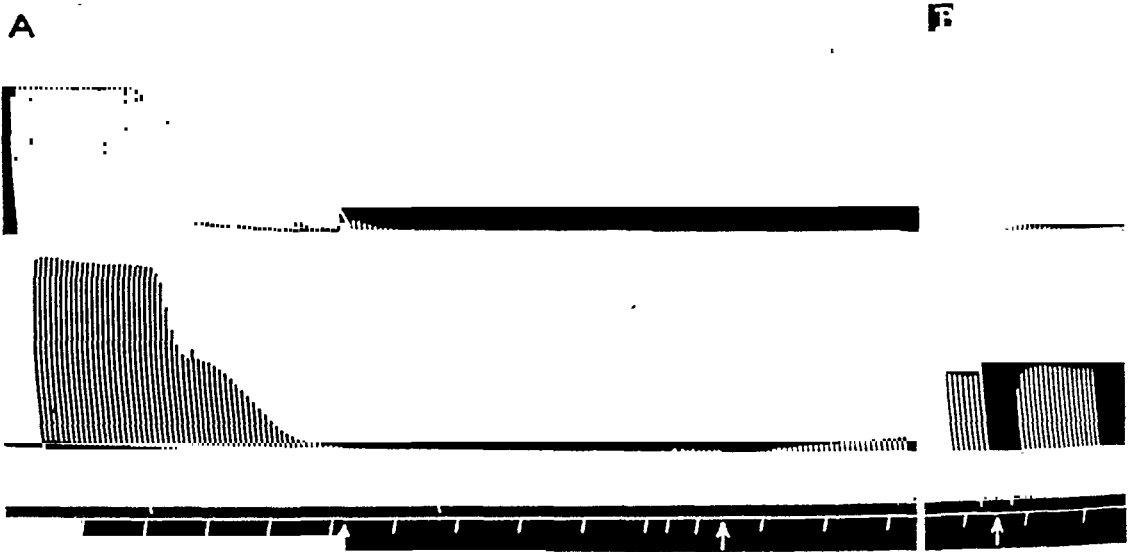
Fig. 2. Records of two soleus muscles, simultaneously stimulated through the popliteal nerves with maximal single shocks every 5 seconds. Upper record, nerve-muscle fresh; lower record, in stage 4.

A. At signal, 0.15 cc. of curare injected intravenously. Time, 1-minute intervals. Three and 9 minutes after the injection (at first and second arrows) the nerves were stimulated 10 and 30 seconds, respectively, at the rate of 60 shocks per second.

B. Twenty minutes after the injection. No response on the fresh side until after tetanic stimulation (30 sec., 60 shocks per sec., at arrow). Good responses of the soleus in stage 4, both before and after temporary inhibition during the rapidly repeated shocks.

stimulation, curare might be expected to have different effects in these various circumstances.

First, two symmetrical muscles (soleus or gastrocnemius), one in the fresh state and the other in the fatigued condition of stage 4, were compared while being stimulated at 5-second intervals with maximal single shocks applied to the popliteal nerve. The intravenous injection of a small amount of curare then showed that the response of the fatigued muscle is depressed sooner than is the response of the fresh one. Furthermore, this earlier effect is followed by a sharper decline in the height of the contractions. These results are shown in figure 2.

Although fatigue at the neuromuscular junction is attended by a prompt indication of the depressive influence of curare, the recovery from curarization begins sooner and is accomplished more rapidly on the fatigued side than on the fresh side. In the experiment illustrated in figure 2, for example, the first slight signs of recovery appeared in the lower record (stage *4*) about 9 minutes after the injection; on the other hand, except as post-tetanic contractions (fig. 2B), they did not appear in the upper record (fresh)
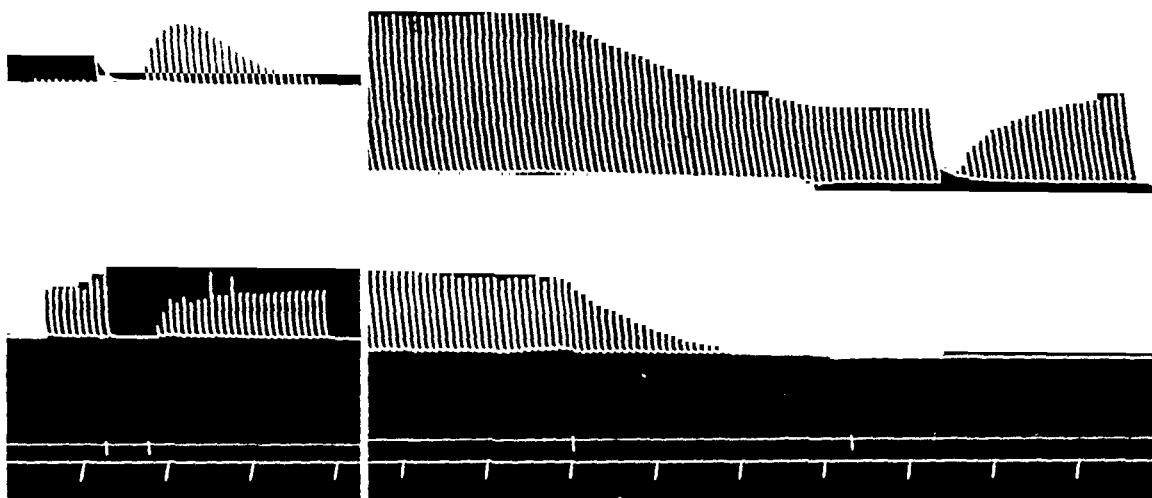
Fig. 3   Fig. 4

Fig. 3. Records of two gastrocnemius muscles simultaneously stimulated through the popliteal nerves with maximal single shocks every 5 seconds. Upper record, nerve-muscle fresh; lower record, in stage *4*. Curare injected 12 minutes previously. Between signals, tetanic stimulation (60 per sec.) for 30 seconds produced Wedensky inhibition. A post-tetanic decurarization occurred on the fresh side only.

Fig. 4. Records of two gastrocnemius muscles stimulated simultaneously through the popliteal nerves with maximal single shocks every 5 seconds. Upper record, in stage *4*; lower record, in stage *5*. At first signal curare (0.05 cc.) injected intravenously; at second signal artificial respiration started. Time, 1-minute intervals. The interruption in the upper record, about 4 minutes after the injection, was due to 10 seconds of stimulation (of both nerves) at 60 shocks per second.

until 31 minutes after the injection. It seems, therefore, that although curare acts earlier at the fatigued neuromuscular synapse, it has there a less profound and less persistent effect, i.e., the fatigued synapse is more resistant than the fresh one (cf. fig. 3).

Another remarkable difference between the performance of a fresh neuromuscular preparation and one in stage *4* is seen in testing for post-tetanic decurarization. A short tetanic stimulation, at high frequency,

applied to a nerve in a curarized animal, will cause temporary decurarization at the nerve terminals (Boyd, 1932). This typical phenomenon does not occur in stage 4. As shown in figure 3 the gastrocnemius in stage 4 (lower record) had partially recovered from curare, while the other gastrocnemius (fairly fresh) was only slightly responsive to the test nerve volleys (5-second intervals). Then a tetanic stimulation, at 60 per second, was applied. Continuance of the single shocks disclosed a striking post-tetanic decurarization on the fresh side, but none on the other side, in stage 4.

Still another difference between the fairly fresh neuromuscular synapse and the synapse in stage 4 is disclosed when, after curarization has been partially recovered from, the nerves are maximally stimulated at a slow rate (2 per sec.). The decline in the degree of contraction as the stimuli are repeated (Wedensky inhibition) is much more marked and more rapid in the fresh than in the fatigued muscle. No definite conclusion can be drawn from this contrast, however, because the rate of recovery from curarization is different on the two sides (see p. 223). The contrast is mentioned here as a matter of record.

A comparison of the action of curare on stages 4 and 5 shows that it is much more depressant on the latter than on the former, i.e., the resistance to curare, increased during stage 4, decreases towards the normal as stage 5 develops. An intravenous dose (0.15 cc.) which does not stop respiration may completely stop the responses to maximal single shocks (delivered at 5-second intervals *via* the popliteal) of a gastrocnemius in stage 5, while merely reducing to about one-half, for a few minutes, the responses of the other gastrocnemius in stage 4, tested in the same manner (see fig. 4). This is a typical phenomenon.

It is worth noting that Wedensky inhibition, produced by slowly repeated stimuli (2 per sec.) after partial recovery from curare, is more prominent in stage 5 than in stage 4. Here again the 5th stage resembles the fresh condition. Comment on the relations of stage 5 to stage 4, however, is made difficult in the circumstances by the differential recovery from curarization in the two conditions.

The post-tetanic increment of contractions in stages 4 and 5. When a series of twitches of skeletal muscle, evoked indirectly by stimuli of uniform intensity, is interrupted by a brief tetanus, the subsequent twitches are greater than the original. Clear evidence is lacking to account for this well-established phenomenon. Further information concerning the conditions which affect its appearance is therefore important.

As shown in figure 5A, a typical augmentation of the single responses occurred after 15 seconds of stimulation of the soleus through the popliteal nerve at the rate of 500 shocks per second. Thereupon the muscle was similarly stimulated for 42 minutes, at the rate of 60 shocks per second,

until stage 4 was well established. Almost immediately after cessation of the fatiguing stimuli, maximal single shocks at 5-second intervals were begun, and at the end of a minute tetanic stimulation (500 per sec.) for 15 seconds was repeated. When the single shocks were continued, the post-tetanic increment failed to appear (see fig. 5B). After a short rest period—4 or 5 minutes—the augmenting influence of the tetanus was again manifest, but not to so great a degree as in figure 5A.

When stage 5 had been developed by further stimulation at 60 shocks per second, the absence of the increment was still more striking (see fig. 5C).
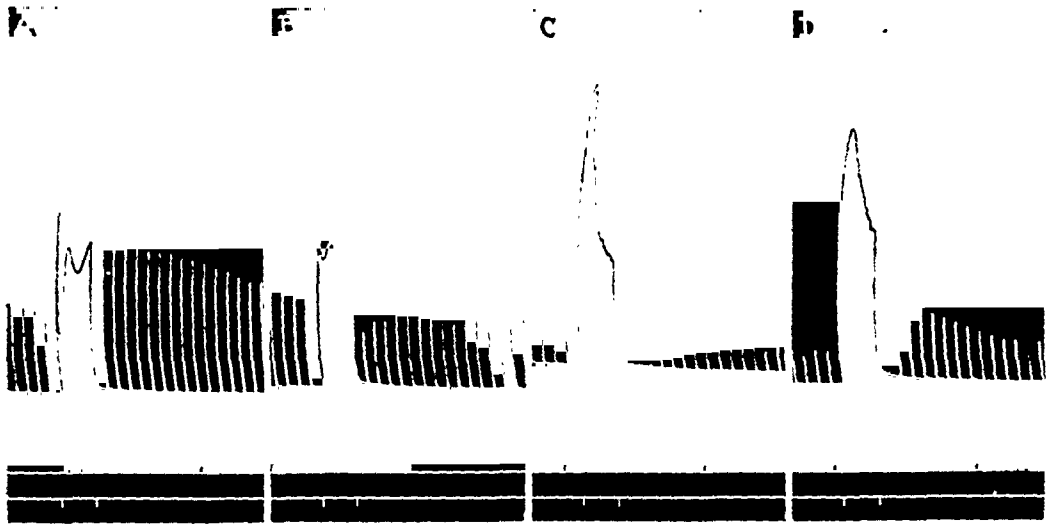
Fig. 5. Records of the right soleus muscle stimulated through the popliteal nerve. Time, 1-minute intervals.

A. Increment of responses to maximal single shocks (repeated at 5-sec. intervals) after 15 seconds of tetanic stimulation at the rate of 500 shocks per second.

B. Absence of the post-tetanic increment when the same test was applied, but after stage 4 had been produced by stimulation of the muscle 60 times per second for 42 minutes.

C. Absence of the post-tetanic increment immediately after interruption of stage 5.

D. Reappearance of the increment, somewhat belated, after 10 minutes of relative freedom from stimulation.

Three tests during 12 minutes after the end of the stimulation which produced stage 5 elicited no post-tetanic increase of response, in spite of two rest periods of 3 minutes each. Ten minutes later, however, a rather belated increase was recorded (see fig. 5D).

Incidentally it may be reported here that our observations have confirmed those of Feng et al. (1938) that the soleus muscle is better suited to manifest the post-tetanic increment than is the gastrocnemius, and that the longer the conditioning tetanic stimulation, within limits, the longer the duration of the subsequent increment.

DISCUSSION. *The effects of prostigmin.* As pointed out earlier, there is indication of a low output of a-ch in stage *4* and of an increased output in stage *5*. The results obtained by use of prostigmin are consistent with that evidence. Prostigmin augments the muscular response in stage *4*, and has chiefly a depressive influence on the response in stage *5* (see fig. 1). In both stages the drug protects a-ch against rapid destruction by cholinesterase. The greater resultant contraction in stage *4* can reasonably be interpreted as due to the participation of a larger number of fibers, brought into action because the persistence of a-ch results in an increased concentration at the synapse. The diminished response in stage *5* is reasonably explained by the development of so high a concentration of the protected a-ch that it reaches up into the paralytic range for many of the fibers (cf. Rosenblueth and Morison, 1937).

Likewise can be explained the influence of prostigmin in shortening the period between the start of stimulation and the first sign of stage *5*, as well as shortening the period of development of stage *5* itself. Stage *5* grows out of stage *4* as a-ch becomes gradually more concentrated in the nerve fibers. By checking destruction of the slowly increasing amount of a-ch in the nerve when the period of fatigue has begun to pass away, prostigmin naturally sets forward the process of recovery.

Rosenblueth and Luco (1939) have shown that there is no correlation between the height of the spike potentials of the stimulated nerve and the development of stage *5* in the responding muscle and thereby they have proved the inadequacy of the electrical theory in explaining stage *5*. That theory also fails to account for the action of prostigmin. Even if prostigmin should have a sensitizing effect which would render electrical impulses from the nerve fibers more effective in stage *4*, that effect could not be reconciled with the depressant action of prostigmin in stage *5*. On the other hand, the theory of chemical transmission, as shown above, can readily elucidate these phenomena.

The effects of curare. Difficulties are encountered in attempting a complete explanation of the quantitative differences in the action of curare at the various stages of neuromuscular transmission. The more prompt depression of response of muscles in stage *4*, as compared with fresh muscles (see fig .2), may perhaps be due to persistent vasodilatation in the fatigued muscle, because of the metabolites of the preceding muscular activity.

On the basis of previous evidence, curare appears to raise the threshold of the muscle to a-ch (see Rosenblueth and Morison, 1937). The diminished height of contractions in figure 2, after an injection of curare, could thus be accounted for. But there is testimony that the a-ch available in stage *4* is less than in the fresh state (Rosenblueth, Lissák and Lanari, 1939), and therefore it might be supposed that curare would be especially effective in that stage. As shown in figures 2 and 3, however, the drug

has a deeper and more persistent depressive action on the fresh neuromuscular junction than on that which has been subjected to prolonged stimulation until it is well fatigued.

Feng et al. (1938) have demonstrated that curare in a small dose abolishes the typical increment of single contractions that follows a brief tetanus, whereas after a larger dose, sufficient to produce a nearly complete synaptic block, the increment reappears. These results were obtained when the neuromuscular junction had not been much stimulated. In our experiments, when the dose of curare was such as to permit a post-tetanic decurarization or increment on the fresh side, the increment did not appear on the side in stage 4 (see figs. 2 and 3). But the muscle in stage 4 was already performing much better than the relatively fresh one. Might it not be possible that the prolonged tetanic stimulation, required to develop stage 4, resulted in a more or less persistent condition resembling that produced acutely and for a short time after a brief tetanus, i.e., the evoking of some adjuvant agent which renders more effective the a-ch liberated by nerve impulses. This suggestion would imply an agent not readily removed or destroyed. It would imply also that the agent is so prominently present, after the prolonged stimulation leading to stage 4, that for a considerable period it could not be increased by brief tetani. Thus the greater resistance to curare of the neuromuscular synpase in stage 4, and the lack of a post-tetanic increment in that stage, could be imagined.

A perplexity arises, however, when this concept is carried over into stage 5. In that stage the neuromuscular junction is more sensitive to curare than that in stage 4 (see fig. 4), and yet it has been stimulated much longer than the junction in stage 4. One way out of the difficulty is to suppose that by the time stage 5 is reached the accessory agent has been exhausted or in some manner rendered ineffective. Then the failure of the increment in stage 4 could result from a maximal presence of the agent, as explained above, and the failure in stage 5 could result from its absence or ineffectiveness. The greater sensitiveness to curare in stage 5 would be consistent with this idea.

The post-tetanic increment in stages 4 and 5. Rosenblueth and Morison (1937) suggested that the post-tetanic increment of the responses to single nerve volleys is due to a diffusion of potassium from the muscle during the period of tetanic stimulation. This suggestion was supported by observations of Brown and Euler (1938) and of Feng et al. (1938). Feng and his collaborators have shown that an intra-arterial injection of a small amount of potassium chloride has all the effects of a tetanic stimulation, and, furthermore, that these effects are modified by eserine and curare just as are the post-tetanic effects. Feng argues, therefore, that the release of potassium at the neuromuscular synapse in an unusual abundance by rapidly repeated shocks would account for all the post-tetanic phenom-

ena. As remarked above, Feng's observations were made on relatively fresh neuromuscular synapses. Admittedly the duration of the increment of responses after a tetanus is too long to be explained by continued action of a-ch—its existence is ephemeral. An accumulation of potassium at the synapse, however, collaborating with a-ch (see Brown and Feldberg, 1936) would furnish the adjuvant agent invoked in the previous section to provide a concept of what might be transpiring in stages 4 and 5. Whether prolonged stimulation, such as is required to bring forth stage 4, would result in a more persistent presence of potassium than results from short stimulation, as was surmised, is not at all evident. And whether a still more prolonged stimulation, that evoking stage 5, would be attended by a great fall in the potassium output, as was surmised, is also not evident. Concerning the possible coöperation of a-ch and potassium at synapses our knowledge is too meager to justify at this time further pursuit of these speculations.

SUMMARY

The muscular response in stages 4 (fatigue) and 5 (recovery while tetanic stimulation is continued) was studied in relation to certain conditioning factors.

Prostigmin has an augmentive action on the muscular response during stage 4 (see fig. 1); a depressive effect is more marked as stage 5 progresses.

Prostigmin advances the onset of stage 5 and accelerates its development (see table 1).

Curare has an early and precipitate depressive influence in stage 4, but the recovery from the depression is sooner and faster than in fresh muscle (see fig. 2); i.e., the fatigued synapse is more resistant to curare than the fresh one.

Post-tetanic decurarization does not occur in stage 4 as it does in the fresh state of the synapse (see fig. 3).

Wedensky inhibition (induced by maximal stimuli applied 2 per sec.) occurs to a more marked degree and at a faster rate in fresh than in fatigued muscle.

In stage 5 conditions are more like those of the fresh state than of stage 4; i.e., the muscle in stage 5 is less resistant to curare than in stage 4 (see fig. 4), and Wedensky inhibition (induced as described above) is more prominent in stage 5 than in stage 4.

The post-tetanic increment of responses to single nerve volleys, which is evident in fresh preparations, disappears in stage 4, if the test is made immediately after the fatiguing tetanus. A short period of rest, however, allows the phenomenon to reappear. The increment is absent also in stage 5, and a longer rest is required for its return than in stage 4. (See fig. 5.)

In the discussion these results are considered in relation to the chemical and electrical theories of neuromuscular transmission.

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THE OXYGEN CONSUMPTION IN VITRO OF BRAIN CORTEX, KIDNEY, AND SKELETAL MUSCLE FROM ADRENALECTOMIZED RATS[1]

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The lowered basal metabolic rate of animals following the removal of the adrenal glands (1) is a striking and constant feature of the changes which take place in all of the species so far investigated. It has not been definitely established whether this reduction is *a*, a specific change resulting from the lack of the adrenal cortical hormone; *b*, a manifestation of an interrelation between the adrenal gland and one or more of the other endocrines known to secrete hormones having a calorigenic action under physiological conditions, or *c*, the indirect result of progressive circulatory and respiratory failure. If the metabolic rate of the whole animal is the sum of the metabolism of all of the tissues plus a certain minimum functional metabolism as suggested by Barcroft (2), then the *in vitro* respiration rate of tissues taken from adrenalectomized animals might yield some information which would help to separate direct and indirect effects of adrenal insufficiency. Either the failure of the direct action of the adrenal cortical hormone on tissue respiration or the secondary failure of action of some other hormone might be expected to affect the *in vitro* as well as the *in vivo* oxygen consumption. However, a lowered basal metabolic rate resulting largely from failure of the circulation, external respiration and other aspects of the minimal functional activities would not necessarily be associated with reduced rate of respiration *in vitro* of the tissues from the same animal.

Field, Belding and Martin (3) demonstrated that the summated tissue respiration in normal adult rats amounts to about sixty-six per cent of

the mean basal metabolic rate, the largest fraction of the remainder being attributed to minimal functional activity. The present communication presents a survey of organ respiration in adrenalectomized animals as part of a study of the relation of summated organ metabolism to basal metabolic rate in adrenal insufficiency.

METHODS. The rats used in this experiment were young adult males and females of the Slonaker-Wistar strain. The range of weight was from 94 to 143 grams. Animals used for comparison were in almost all cases litter mates. For eight to ten days preceding the beginning of each experiment the animals were kept in a warmed room under the same conditions of temperature and alimentation as were to be encountered after adrenalectomy.

After this preliminary acclimatization the animals were bilaterally adrenalectomized under pentobarbital anesthesia (37 mgm. per kgm. intraperitoneally) through the lumbar approach. A second series of animals was subjected to similar acclimatization and surgical procedure except that the adrenal glands were left *in situ* after stripping the pedicle and dissecting the gland free of the kidney.

Two groups of ten animals each were adrenalectomized at a sufficient interval to permit sampling over the later portion of the average survival time for adrenalectomized animals in this colony. The series were arranged to begin tests on the fourth post-operative day, and to include duplicate organ samples from two or more animals over fourth to eighth post-operative days inclusive. The respiration rates of the organs of the adrenalectomized animals were compared with the rates for the same organs on the same days after operation in the dummy operation control series.

The technique for the preparation of tissues and the measurement of rate of oxygen consumption has been described in previous communications (3, 4). The only deviations from the previous procedure include changes in method of preparation of brain cortex and the obtaining of ratios of the wet weight of tissues to their dry weight. In place of free-hand cutting of cortical material with small scissors, we obtained thin slices of cortex by pressing a small fiber template down upon the surface of the brain and shaving off with a razor blade the cortical tissue which was caused to bulge through the opening in the template. In this way it was possible to obtain thin uniform slices of relatively larger area and thus restrict trauma to a smaller proportion of the tissue taken for use in the Warburg vessels.

The ratios of the wet weight to the dry weight of tissues were derived from samples of organs taken at the time the slices were prepared for measurement of oxygen consumption. The organs were prevented from drying during sampling by keeping them at room temperature in petri dishes

lined with filter paper moistened with Dickens-Greville (5) Ringer's solution.

The wet weight of organ slices was obtained by direct weighing on a torsion balance, some slices were transferred to crucibles and dried to constant weight and others were placed in the respirometer vessels.

RESULTS. 1. *Relative water content.* Table 1 presents the ratios of the wet weight to the dry weight of the organs studied in the adrenalectomized animals and the control (dummy operation) animals from the fourth to the eighth post-operative day inclusive.

The ratio becomes larger with time in the organs taken from the adrenalectomized animals. In the case of kidney and skeletal muscle the most striking change occurs sharply between the fifth and sixth day, while in

TABLE 1

Wet weight/dry weight ratios of brain, muscle and kidney

POST-OPERATIVE DAY	NUMBER OF ANIMALS		BRAIN		MUSCLE		KIDNEY	
	Controls	Adrenal-ectomized	Control	Adrenal-ectomized	Control	Adrenal-ectomized	Control	Adrenal-ectomized
4	2	2	5.59	5.13	4.61	4.49	5.09	4.55
5	2	4	5.85	4.81	4.91	5.13	4.45	5.56
6	2	4	5.12	5.23	4.56	5.67	4.82	5.23
7	2	4	5.78	5.56	4.83	5.18	5.05	5.32
8	2	2	5.05	5.97	4.67	5.46	4.97	5.66
Mean.....................			5.48	5.34	4.72	5.19	4.88	5.26
Standard error...........			±0.17	±0.20	±0.07	±0.68	±0.12	±0.86
P......................			0.5000+*		0.0170		0.1276	

* Greater than 0.5000.

brain the change appears to be somewhat more gradual. The control animals show no significant change in the ratio throughout the time period. The mean of the ratios for brain over the entire period show no significant difference between the adrenalectomized animals and the controls. The difference observed in the muscle taken from the two series indicates that there is an increase in total water of this tissue. The same comparison for kidney indicates no significant change. It must be remembered that the amount of water present in the kidney as glomerular filtrate or formed urine is capable of influencing the wet to dry ratios to a marked extent. Adrenalectomized animals show a progressive reduction in urine volume, and it follows that one might expect the kidney of an animal with intact adrenal glands to contain a relatively larger proportion of water present as glomerular filtrate or as urine than the kidney of

an adrenalectomized animal. If the factor of urinary water content were equalized in the adrenalectomized and the control animals the ratios might then differ more widely in such a way that the difference would indicate a much larger proportion of tissue water in the kidney of the adrenalectomized animal. In the absence of such correction the difference observed may be deceptively low.

2. *Organ respiration on wet-weight basis.* a. *Brain.* The data presented in table 2 indicate that the respiratory rates of brain slices from the two groups are not significantly different. No tendency of the respiratory rate to decline with time in any given period of measurement was observed.

b. *Skeletal muscle.* It was found that the respiratory rate of skeletal muscle did not differ in the control and experimental animals until the seventh and eighth days after operation, at which time muscle from experimental animals showed a somewhat lower rate of oxygen consumption than corresponding muscles from the controls. The difference between the means for the entire period yields a P value of 0.3232 which is clearly not significant (7).

An analysis of the same results was made so as to separate the initial three days of the post-operative period studied from the last two days. In spite of the absence of a sufficient depression of respiration to account for a significant difference between the mean rates over the entire interval studied, it is demonstrated that the fall in respiratory rate of muscle during the last two days (P = 0.0098) is sufficient to justify the assumption that the difference is a real one.

c. *Kidney.* The difference in respiratory rate between kidney slices from control and adrenalectomized animals is more striking than in the case of muscle. Following the fifth day after operation the rate of kidney respiration is reduced by more than fifty per cent as compared with the controls. The magnitude of this decline during the last three days of the period is sufficient to account for a significant difference (P = 0.0590) in the mean rates over the entire five days studied.

Separate comparison of the first two and the last three days after operation reveals no difference during the fourth and fifth days, but the comparison of the latter portion yielded a P value of 0.0056, or about six chances in a thousand of such a difference's being encountered through errors of sampling.

3. *Organ respiration on dry-weight basis.* The increase in the ratio of the wet weight to the dry weight seen in the organs of adrenalectomized animals suggests that a part, at least, of the decline in respiratory rate observed when measurements were expressed on the wet-weight basis might be associated with the increased water content of the tissues.

When the same values for oxygen consumption are expressed on a dry-

weight basis, it is noted that the differences observed on the wet-weight basis have almost entirely disappeared. The only statistically significant difference (fewer than five chances in one hundred) is seen in the comparison of the respiratory rate of kidney during the last two days of the period. If the ratios of wet weight to dry weight for kidney tissue did

TABLE 2A

Q_{O_2} on wet-weight basis

POST-OPERATIVE DAY	CONTROLS				ADRENALECTOMIZED			
	Number of animals	Brain	Muscle	Kidney	Number of animals	Brain	Muscle	Kidney
4	2	1.81	0.58	3.86	2	2.00	0.50	3.73
5	2	1.70	0.54	3.27	4	1.80	0.57	3.50
6	2	1.72	0.43	3.49	4	1.85	0.56	2.71
7	2	2.04	0.54	4.11	4	1.85	0.40	2.21
8	2	1.78	0.55	3.60	2	1.78	0.37	2.30
Mean......		1.81	0.53	3.66		1.86	0.48	2.89
S.E. $\overline{X}$....		±0.061	±0.025	±0.146		±0.038	±0.039	±0.311

* Q_{O_2} = cu. cm. O$_2$/gm./hr.

TABLE 2B

Q_{O_2} on dry-weight basis

POST-OPERATIVE DAY	CONTROLS				·ADRENALECTOMIZED			
	Number of animals	Brain	Muscle	Kidney	Number of animals	Brain	Muscle	Kidney
4	2	10.1	2.7	19.6	2	11.0	2.4	17.8
5	2	10.0	2.6	14.5	4	9.2	2.6	15.9
6	2	8.8	2.0	15.1	4	8.9	2.9	15.1
7	2	11.8	2.6	20.7	4	9.7	2.3	11.6
8	2	9.0	2.5	17.9	2	9.9	1.9	12.3
Mean......		9.9	2.5	17.6		9.7	2.4	14.5
S.E. $\overline{X}$....		±0.54	±0.13	±1.22		±0.33	±0.15	±1.18

not suffer from the error introduced by the difference in urinary water the values should yield an even smaller difference.

It is interesting to note that in brain, in which no difference in relative water content was noted, there appears no "decrease" of respiration when the oxygen consumption is expressed on the wet-weight basis or the dry-weight basis over the entire period of insufficiency studied. This observation is in substantial agreement with Tipton's recent report (8).

DISCUSSION. The decline of basal metabolic rate, expressed on a calculated surface area basis, of adrenalectomized animals begins usually a little more than half way through the survival period of insufficiency (1) and falls from 20 per cent to 50 per cent below the pre-operative value.

The observed lowering of respiration in both kidney and muscle calculated on the wet-weight basis is in rough agreement with the magnitude of the change reported for the intact animal and is not strikingly different in its time course. However, when the *in vitro* respiration is calculated on the basis of dry weight, the magnitude of the difference between the experimental group and the controls becomes insignificant with the exception of that of kidney, in which the observed difference would be encountered only three times in one hundred in random sampling of a series in which no real difference existed.

This observation indicates *a*, that the lowered basal consumption seen in adrenalectomized animals is not entirely the result of failure of minimal functional activity, and *b*, that the altered water distribution can account for the change observed in the *in vitro* respiration of brain and muscle, and in part for the change in respiration of kidney when the results are expressed on the wet-weight basis.

The prime importance of relative water content in determining the direction of change in respiration described is entirely consistent with the cellular hydration reported in adrenal insufficiency (9, 10, 11) and in similar electrolyte imbalances induced by intraperitoneal injections of glucose (12), or direct alterations of the sodium and chloride concentration of the extracellular fluid (13).

Under the conditions of these experiments, the influence of secondary factors, consequent upon water and electrolyte changes is markedly reduced. After the transfer of the organ slices to Dickens-Greville Ringer's solution, a large portion of the water weighed out with the sample becomes metabolically ineffective *in vitro* because it is no longer in the cell.

Field, Belding and Martin (4) in discussing the absence of significant difference between variability of Q_{O_2} on wet and dry bases state "an important theoretical consequence is that tissue water content is related as closely, in a statistical sense, to the factors determining Q_{O_2} as is the miscellaneous aggregate of substances making up dry weight." The findings in the present experiment emphasize the validity of this claim. It is only required to point out the necessity of restricting the use of the wet-weight basis to situation in which there is no possibility of a shift of water into the tissues and further, the desirability of employing a medium in the *in vitro* situation which will imitate as closely as possible the environment of the cells *in vivo*.

The decrease in rate of respiration of kidney tissue which was demonstrated on both the wet and dry weight bases is interesting in connection

with the important rôle played by this organ in the secondary changes of adrenal insufficiency. Since the respiratory rate of kidney is the highest per unit weight of any tissue in the body it is not remarkable that interference with oxidative metabolism through a defect in phosphorylation (15) or in other ways (6) would be manifested first and most profoundly in the kidney. In tissues having a slower respiration the result of interference with metabolism in the same way may be so small in magnitude as to be obscured by the consequences of the changes in electrolyte pattern induced. It may be suggested that the only way clearly to demonstrate the direct effect of lack of adrenal cortical hormone on the metabolism of tissues other than the kidney would be to protect them from intercurrent injury and secondary influences until sufficient time has elapsed for the specific deficiency to be manifested.

SUMMARY

1. The *in vitro* respiratory rates and the ratios of wet to dry weight of skeletal muscle, kidney, and brain from adrenalectomized rats and control animals subjected to a dummy operation of equal severity have been compared over the period from the fourth through the eighth day following operation.

2. Increases in water were found in muscle from adrenalectomized animals. The changes in the kidney are variable and those in brain insignificant.

3. The decrease in respiratory rate expressed on a wet-weight basis which was noted in kidney after the fifth day following adrenalectomy persisted but was found to be smaller in magnitude when the same rates were expressed per gram dry tissue. The differences observed in muscle respiratory rate disappeared when the rates were calculated on the dry-weight basis.

4. The respiration of brain does not change significantly.

5. It is suggested that:

a. The significant reduction of rate of respiration, based on the dry weight, of kidney from adrenalectomized animals may be causally related to the high rate of respiration of this organ.

b. The use of the wet weight as a basis for expressing results of *in vitro* studies of organ metabolism should be restricted to situations where there is no possibility of a shift of water from one "compartment" of the tissues to another.

c. A clear demonstration of the direct effect of absence of the adrenal cortical hormone on the metabolism of tissues other than the kidney is possible only under conditions which protect those tissues from intercurrent injury and secondary effects of deficiency until sufficient time has elapsed for the specific deficiency to be manifested.

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THE ACTION POTENTIALS OF THE SQUID EYE

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In the cephalopod eye, containing only sensory cells, the electroretinogram (ERG) has been found to consist of a single sustained potential (Beck, 1899; Piper, 1904; Fröhlich, 1913). Brücke and Garten (1907) and Piper (1911), considering the inversion of the vertebrate retina, identified this monophasic action potential on an electrical basis with the "positive" action potential (i.e., the *b*-wave or component PII; Granit, 1933) of the vertebrate eye. The analogy thus would require PII to be localized to the receptorial layer of the vertebrate retina, a suggestion already made by Kühne and Steiner (1881). However, in view of the complex structure of the vertebrate eye, an attempt to identify components of the vertebrate ERG with the receptorial action potential of the cephalopod eye should be made not only on an electrical basis (cf. Therman, 1938; Granit and Therman, 1938) but at the same time also by a comparison of the effects of different biological agents.

Besides a general investigation of the nature of the squid ERG, the present paper deals with a study of the effects of glucose, potassium, atropine and adrenaline, as well as oxygen, on the ERG of the cephalopod eye (*Loligo pealii*).

It is worthy of mention that earlier electrophysiological work on the cephalopod eye has been done chiefly on *Eledone moschata*, and Fröhlich (1913) reports that he did not find the eye of *Loligo* as useful as that of *Eledone*. However, the anatomical structure of the eye in both species is essentially the same (Grenacher, 1886; cf. also Hess, 1905; Hanström, 1928).

METHOD. The squids were kept in running sea-water in a dimly illuminated basin. A number of experiments were also carried out on squids kept in light or in complete darkness. A 200-W electric bulb was used as a source for the stimulating light, which passed through a condenser lens and a water-filter. The strongest intensity used (I/1) was 300 Lux. By means of Wratten neutral-tint filters the intensity could be

varied between I/1 and I/10,000. The light was thrown into the whole eye by a mirror above the eye-holder. The time and length of exposure was controlled by a photographic shutter. The length of the exposure in routine experiments was 0.25 to 0.5 sec. and the interval between each stimulation 2 to 5 min.

As several electrodes were placed at the same time on the eye, it was found convenient to use plain chlorided silver wires. Photo-electric effects were constantly watched for and were kept below 10 μV.

The recording equipment consisted of two condenser-coupled amplifiers in connection with a two-channel ink-writer, thus enabling simultaneous records to be taken from two different points. The time constant used (halfway down in 0.7 sec.) was long enough to reveal the essential features of the ERG of short exposures. However, it did not permit observations of a slow process like the PI component (c-wave) in the vertebrate eye. The frequency characteristic of the recording system should be considered as also limiting the possibilities of recording action potentials of a duration comparable to axon spikes.

RESULTS. The electrical activity of more than 80 eyes from different squids (*Loligo pealii*) has been studied. For a general schematic view of the electrode placements as well as the relative size and polarity of the responses see figure 1 and legend.

On the whole no pronounced electrical activity could be revealed by any combination of electrodes E_3, E_4, E_5 and E_6. A small positive action potential was often recorded at E_3. Its size fluctuated with the size of the positive deflection recorded with E_2 (see below) and was also dependent on the distance to the fundus of the eye, being smaller the longer the distance. Records taken with E_5 connected to E_6 gave in a few cases a faint sustained negative potential. In one single case it remained for more than one hour and was of the order of 10 to 40 μV (see fig. 4), while in the other cases it soon disappeared. This rare occurrence of a weak electrical activity in the ganglion or its optic nerve is in sharp contrast to the bigger and easily obtained potentials from the eye-ball.

The ERG. It was soon found that in the case of a dark-adapted animal the weak light (red or white) necessary for a successful enucleation was strong enough to depress the size of the action potential. The subsequent enhancement is comparable to the effect of the regeneration of visual purple on the ERG of the frog's retina. This resemblance is also emphasized by the occurrence of a red color in the squid retina after being kept in darkness for one-half to one hour. It is found that the enhancement of the potential is facilitated when the eye is opened (cf. Granit and Munsterhjelm, 1937).

Provided that moderate intensities are used, a monophasic, sustained, positive action potential is recorded when one electrode, E, is placed on

the surface of an intact eye and the second electrode, E_6, as reference point, on the ganglion (cf. fig. 1, A). The shape and size of the action potential does not depend on whether the second lead is on the ganglion or *Nervus opticus*, nor will an appreciable change occur when the *Nervuli optici* are crushed. The size of the potential, however, is largely de-
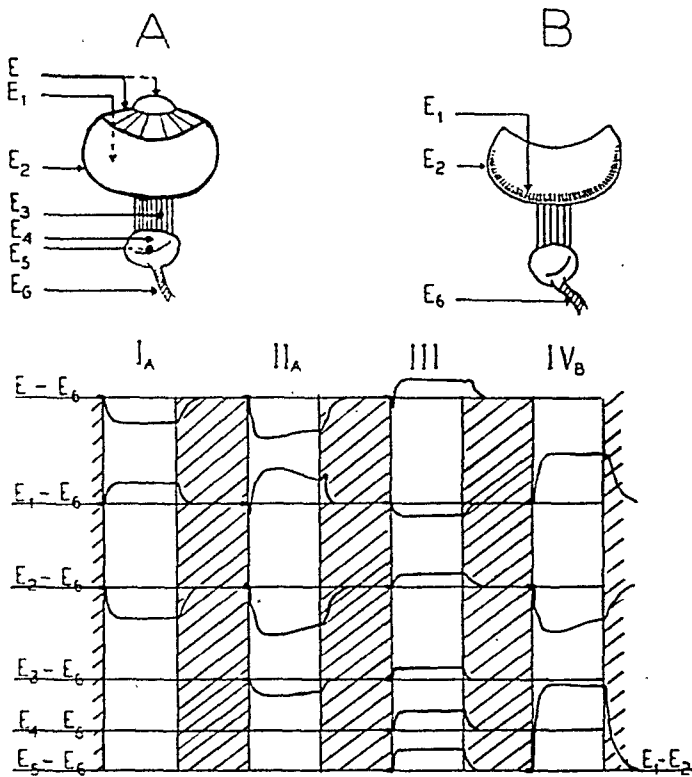
Fig. 1. Schematic view of the electrode placement in an intact (A) and an opened (B) eye as well as corresponding records obtained with indicated electrode combinations. Of these electrodes (corresponding to A) all but E_1 and E_5 were surface electrodes. E_1 and E_5 consisted of thin chlorided silver wires insulated to their tip and were inserted into the tissue. Upward deflection indicates negativity at the low-numbered electrode. The intensity of the stimulus in group I_A and IV_B was I/100, in group II_B I/10. Time of exposures, 0.25 sec.

Group III contains records representing observations of "atypical" action potentials assembled from different experiments. For their relationship to the regularly obtained deflections reproduced in the groups I_A, II_A and IV_B see text.

pendent on the placement of the eye-electrode. The biggest response is often obtained from the scleral part, the smallest, from the lens.

If strong intensities are used (e.g., I/10; cf. fig. 1, II_A, $E - E_6$) and the eye has been kept in darkness for some time, the positive action potential recorded is not smooth. At both "on" and "off" there are indications of an interfering action potential of opposite electrical sign. However, these irregularities are more commonly seen if the eye has been damaged

in some way. In fact, pure negative action potentials may be obtained if the "active" electrode is in contact with the ocular fluid (see fig. 1, A, $E_1 - E_6$; III, $E - E_6$). These results indicating the presence of two action potentials of opposite sign with regard to a common reference point on the optic ganglion were confirmed and more clearly brought out by using opened eyes (fig. 1, B). With regard to the reference electrode, E_6, the electrode on the receptorial layer, E_1, revealed a negative action potential while E_2 at approximately the same time became positive. The relative size of these two action potentials may be changed by certain factors such as state of adaptation, strength of stimulation, effect of drugs, or impaired condition of the eye.

In light-adapted eyes the negative action potential (at E_1) is usually lacking and only a positive action potential of moderate size may be recorded from the outer surface of the eye, E_2, or from the exposed peripheral side of the retina. However, if the eye is kept in darkness a negative action potential at E_1 gradually appears. At the same time the positive action potential increases also. This gradual increase of both action potentials continues during the next 30 to 60 min. but follows different curves (fig. 2). Again, long exposures or frequent stimulation exhaust more rapidly the negative action potential than the positive one. At the end of the enhancement of the action potentials, the records obtained with strong intensities and an electrode inside the eye, E_1, usually show a positive deflection preceding the negative action potential (fig. 1, II$_A$; $E_1 - E_6$). This positive deflection may persist as a monophasic positive action potential irrespective of the disappearance of the negative action potential and show the same characteristics as the positive action potential recorded at E_2. However, the size of such positive deflections recorded at E_1 are always much smaller than the corresponding potential at E_2. A similar "spread" of the negative action potential is indicated by the above mentioned irregularities at "on" and "off" of the positive surface potential, E_2. The positive action potential, E_2, has never been seen preceded by a negative deflection.

The positive action potential responds with deflections of greater differences in size within a certain range of intensities than the negative one. The following figures were obtained simultaneously for the negative and positive deflections from the same eye stimulated with two different intensities; I/100: pos. 20 μV, neg. 20 μV; I/10: pos. 70 μV, neg. 45 μV. The positive action potential thus was increased 3.5 times, while the negative was increased only 2 times.

If the electrical activity of the retina is recorded between the electrodes E_1 and E_2, instead of using a common reference electrode, E_6, a smooth action potential is obtained. Its polarity *always* indicates negativity at the end of the receptors (i.e., *Membrana limitans*) and positivity at the

base (fig. 1, IV$_B$, $E_1 - E_2$). The size of this deflection is always bigger than the "individually" recorded potentials (IV$_B$) and may be even bigger than their sum.

Effect of chemical agents. The effect of different chemical agents emphasizes the relative differences between the negative and positive action potentials. Such differences occur both with respect to the time of onset as well as to the degree of the effects.
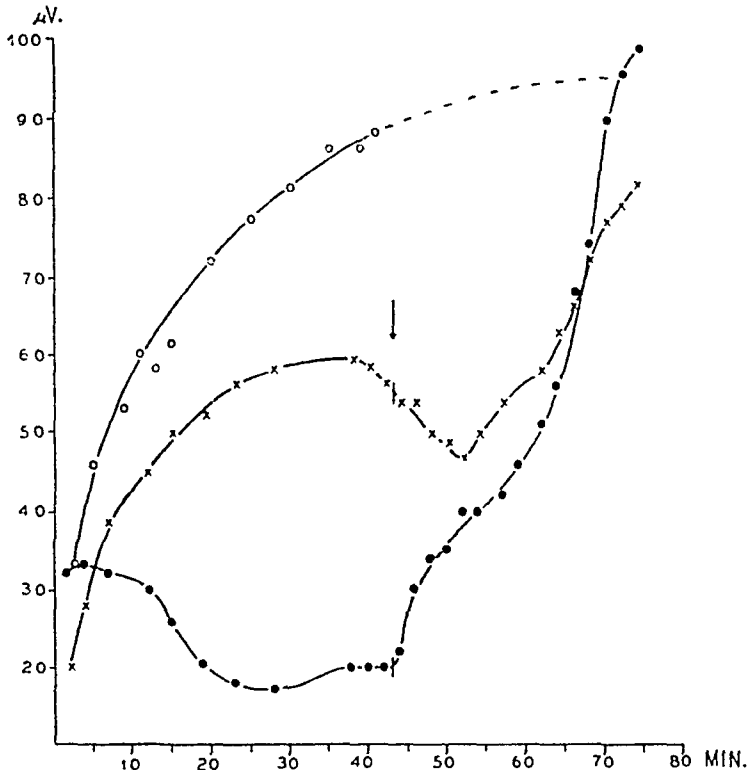
Fig. 2. Eye previously light-adapted. The curves show subsequent increase of the negative (dots) and positive (crosses) action potentials. Glucose was given at the moment indicated by arrow. For comparison a curve is drawn of a "normally" increasing negative action potential (circles) obtained from another animal. Note correspondence in the starting and end points of the two curves (dots and circles).

Glucose (isotonic with sea-water) enhances both the negative and the positive deflections but the maximal effect occurs at different times and to a different extent, as is seen in figures 2 and 3. Both potentials may maintain their final size for several hours. A second drop of glucose is without any appreciable effect. With regard to the negative action potential the glucose effect is most striking in a previously light-adapted eye which has been kept in darkness without showing any significant increase of this potential. This is shown in figure 2, where the regeneration of the positive (crosses) and negative (dots) action potential is seen before and

after a glucose treatment. For comparison a regeneration curve (circles) of the negative action potential in another eye under optimal conditions (without glucose) is drawn in the same diagram.

If a light-adapted eye is filled with glucose immediately after the enucleation, a smooth increase of both positive and negative action potentials is obtained. The rate of increase as well as the final size of the deflections is directly comparable with that obtained in the best experiments without a previous treatment with glucose.
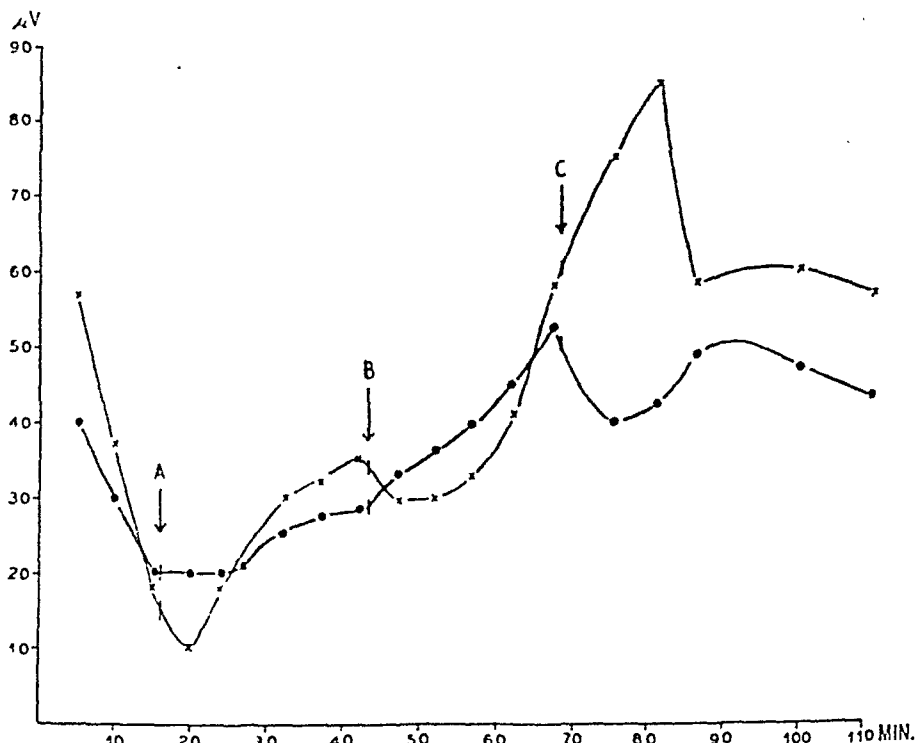
Fig. 3. The effects on the negative (dots) and the positive (crosses) action potentials by successive treatments of a previously light-adapted eye with oxygen (at *A*), glucose (at *B*) and adrenaline (at *C*).

In another series of experiments, a continuous stream of pure oxygen was led directly into the dark-box and the favorable effect obtained resembles that of glucose. However, in most of the cases the positive action potential is affected far more than the negative whereas the opposite is true for the glucose effect. A subsequent treatment with glucose is thus usually effective in increasing the negative action potential.

The solvent for the different potassium concentrations, ranging between 0.01 and 10 per cent, has been mostly glucose. After the subsidence of the glucose effect a potassium-glucose solution was added. Generally both the negative and the positive action potentials show a great resistance

to treatments with potassium solutions. Concentrations below 0.5 per cent were hardly effective at all. Clearer effects were obtained only with stronger potassium solutions. A facilitation of both the positive and negative action potentials (particularly the positive) may be obtained with 0.5 to 2 per cent potassium solutions (fig. 4). With still stronger potassium solutions (2 to 10 per cent) a decrease of both action potentials
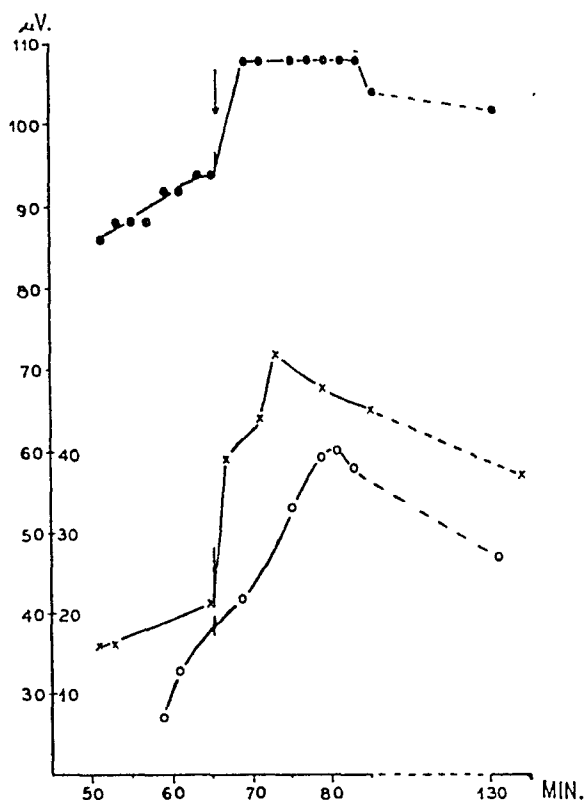
Fig. 4. The effect of potassium on the retinal action currents and the ganglionic action potential. Negative action potential (electrodes $E_1 - E_6$, fig. 1; IV_B) = dots; positive action potential ($E_2 - E_6$) = crosses; ganglionic action potential ($E_5 - E_6$, group III) = circles. The eye, previously treated with glucose, was filled with a 1 per cent KCl solution (dissolved in glucose) at the moment indicated by arrow. The size of the ganglionic action potential corresponds to the inside scale.

is generally observed, but, while the negative deflection declines more and disappears from the record, the positive one, though decreased, still may remain.

The effect of adrenaline (dissolved in glucose) consisted of a slow depression of both action potentials, as seen in figure 3. This depressant action does not affect appreciably the shape of the deflections.

The effect of atropine (1 per cent dissolved in glucose) resembles the effect of adrenaline. A marked facilitating effect of atropine on either action potential has not been observed.

DISCUSSION. The reason that a ganglion action potential has been so rarely seen, as well as its early disappearance compared with the resistant action potentials of the eye, may be found in structural and functional differences between receptors and ganglion cells, the latter being more susceptible to removal from the natural environment. The small positive action potential sometimes recorded from the *Nervuli optici* is obviously due to an electrotonic spread (cf. Eccles, 1935) of the main action potential originated in the retina.

Considering the fact that during illumination two action potentials of opposite electrical sign (with regard to a reference point) can be recorded from opposite sides of the receptorial layer of the squid's retina, the question arises whether these potentials actually represent bio-electrical signs of two different processes, or whether the difference in polarity is merely due to the electrical pathways involved and we thus are recording one and the same process. On account of general observations on the effect of a proceeding dark-adaptation and intensity-variation it can be tentatively concluded that the two action potentials are two different processes. This suggestion is supported by the differential effects of glucose, oxygen, potassium, adrenaline and atropine. If the two action potentials were due to a common process and thus merely signs of a "dipole" electrical system it would be reasonable to expect a closer agreement in their mutual variations. Furthermore, the presence of two retinal action potentials in the squid eye may easily explain the old differences in opinion about the polarity of the cephalopod ERG (cf. Beck, 1899; Piper, 1904).

From the electrical point of view, it may be emphasized that the potential distribution found (positivity at the base and negativity at the end of the receptors) never is completely reversed, and that the two action potentials add and give a maximal smooth ERG if the electrodes are on opposite sides of the retina. The fact that a small negative deflection may, in the absence of the positive action potential, be recorded from the outside of the eye,—or *vice versa* a small positive deflection may, in the absence of a negative action potential, be recorded from the inside of the eye (cf. fig. 1, III)—seems also to indicate that the two action potentials are due to different processes. With regard to the placement of the electrodes with a common reference electrode on the ganglion, the positive action potential should be expected to counteract the recording of the negative action potential. The "true" size of the negative action current can be approached only by subtracting the positive deflection from the combined electrical activity obtained in an electrode combination of $E_1 - E_2$ (fig. 1, IV_B). According to this view a main increase of the positive action potential should also be revealed as a decrease in the recorded negative deflection (provided that the actual size of the latter

is unchanged). Such opposite changes are commonly seen and demonstrated in figures 2 and 3. It is also interesting to note that the positive deflection seems to be generated faster than the negative as is indicated by the appearance of a positive "notch" preceding the negative action potential (cf. fig. 1, II_A, $E_1 - E_6$). A similar positive "notch" preceding the main retinal action potential of *Eledone moschata* has been reported by Fröhlich (1913).

In his elaborate study of the histology of the cephalopod retina, Grenacher (1886) has pointed out that the cephalopod retina possesses only rods and that the picture reveals essentially the same details as are found in rods of amphibians and mammals. Three parts of the rod are distinguishable: the outer and inner segments and the nuclear part. It is generally believed that these parts are structural differences within a single cell (cf. Detwiler, 1938), although a different opinion exists (cf. Moroff, 1922). The inner segments have been assumed by Kühne and Steiner (1880) to possess electrical activity during illumination, whereas Garten (1907) in this respect emphasized the importance of the outer segments. However, at that period the suggestion referred only to the *b*-wave (PII) of the frog's ERG.

In general the various agents used in this investigation affect both action potentials of the squid eye in the same direction. Neither agent has been shown to elicit a clear enhancement of one action potential while the other is being depressed. In the frog eye weak concentrations of a potassium solution are sufficient to depress or remove the PII component while PIII even by stronger solutions is unaltered or slightly increased (Therman, 1938). In the squid eye both the negative and positive action potentials are strikingly resistant to a treatment with potassium and may even show an increase in size. The specific effects of adrenaline and atropine on the PII component of the frog ERG (Therman, 1938) have not been observed in the squid eye. These results inevitably suggest an identification of both the negative and the positive action potential of the squid eye with the PIII component of the frog eye. Irrespective of the close resemblance in shape of the combined action potentials of the squid eye ($E_1 - E_2$, fig. 1, IV_B) and the PIII component in the vertebrate eye, it remains to be seen whether PIII also involves two electrophysiological processes.

Electrophysiologically PIII is the first response to be seen in a vertebrate eye at the moment of light-stimulation, but, also, ontogenetically this component is the first to occur in a developing rat's retina (Keeler *et al.*, 1928). This elementary character of the PIII component is emphasized by the identification of PIII with the receptorial action potentials of the squid eye.

SUMMARY

The electrical response of the squid eye (*Loligo pealii*) to illumination has been recorded from different parts of the eye-bulb and ganglion by means of two condenser coupled amplifiers connected to a two-channel ink-writer.

A faint, subsiding action potential has been recorded occasionally from the optic ganglion.

Two monophasic, sustained action potentials of opposite electrical sign (with respect to a common reference electrode on the optic nerve) have been recorded from the retina. A negative deflection is recorded from the end part and a positive from the basal part of the receptors. In bipolar recording with electrodes on opposite sides of the retina the potential obtained is approximately the sum of the two action currents and the polarity indicates negativity at the end and positivity at the base of the receptors.

The two action potentials are in general affected by a progressive dark-adaptation, oxygen, glucose, potassium, adrenaline and atropine in the same direction but to a different degree and at different times. Both the negative and the positive action currents (and thus their combined electrical activity) resemble in shape and reaction the PIII component of the vertebrate eye.

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THE GLUCOSE UTILIZATION OF HEPATECTOMIZED DIABETIC RABBITS

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The determination of the glucose consumption rate of the extra hepatic tissues of the depancreatized animal is of first importance in solving the problem of diabetes. Such a determination also throws light on the question of conversion of fat to carbohydrate in this disease. One must remove the liver to carry out a glucose balance in any animal since this organ at all times is adding an indeterminate amount of sugar to the blood. Removal of the gastro-intestinal tract is also necessary if the animal has been recently fed since then absorption of glucose from the tract is a complicating factor.

The principle of the method is simple. Diabetes is produced by pancreatectomy and some time later the liver is removed and one determines the rate at which glucose must be injected intravenously to maintain the blood sugar constant at the preoperative level. The amount of sugar excreted by the kidneys during the determination must be subtracted from the amount injected to give the net injection rate. One infers then that the tissues are removing the glucose from the blood stream at this net rate and one assumes that the tissues had been behaving in the same way prior to the operative removal of the liver.

Mann and Magath (3) showed that the blood sugar of the diabetic dog fell after hepatectomy, but did not measure the glucose utilization rate. Yater et al. (4) made such a quantitative study. They injected glucose at a rate sufficient to maintain the blood sugar constant and found that the normal hepatectomized dog used glucose at the rate of 250 mgm. per kilo per hour and the hepatectomized diabetic dog at 190 mgm. per kilo per hour. There are definite objections to the conditions of their experiments. The kidneys were removed; this has been shown to increase glucose disappearance (1). No mention is made as to whether the animals were fasted or not; this too has a bearing on the results (5). Furthermore these authors state as does Mann (6) that the depancreatized dog does badly after hepatectomy and rapidly becomes moribund. Anyone familar with glucose utilization determinations in hepatectomized animals is

aware of the marked effect that poor general condition may have on the rate of glucose disappearance, usually increasing it. In the final breakdown of the animal, commonly referred to as "the second stage after hepatectomy", the glucose requirement is markedly increased (7). If the diabetic condition causes the onset of the second stage to come on prematurely in dogs, this would tend to cause a high glucose requirement. Besides this, we have observed that the development of any symptoms indicating that the physiological processes of the body are not altogether normal is often accompanied by an increase in glucose need. This is illustrated by the results in a group of hepatectomized and eviscerated rabbits which we had eliminated from other series and not reported,
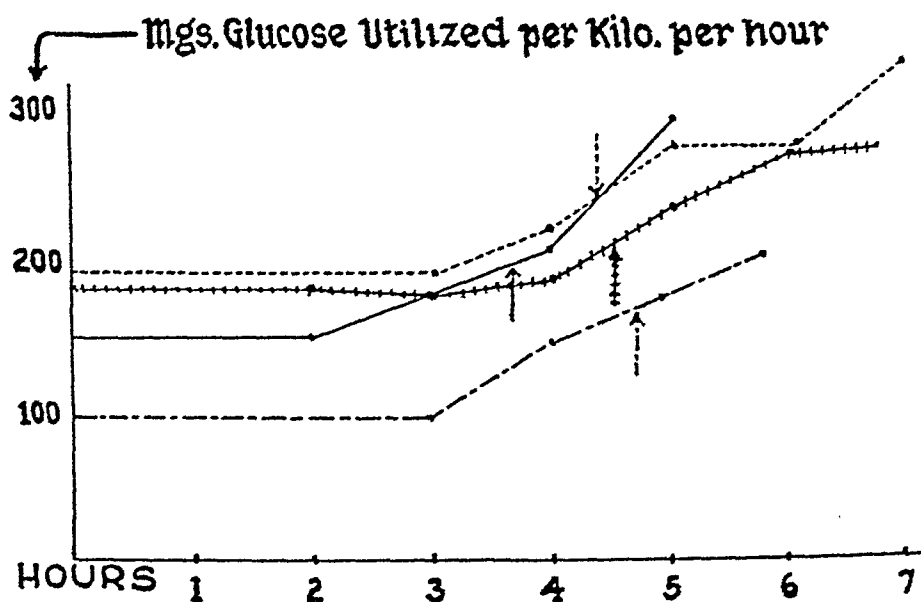
Fig. 1

because the condition of the animals was unsatisfactory and not in keeping with the requirements which we have stipulated for such experiments (5). For these animals the note "head drooping" (indicating weakness or poor righting reflexes) was put into the record by the observer without his having had any regard to the glucose utilization rate at the time. In figure 1 are depicted the results in these animals showing the increase in glucose utilization which develops after a few hours. The arrows indicate the time when the note was made regarding the condition of the animal (head drooping). It is quite obvious that the glucose utilization increases as the condition of the animal becomes worse. These results are to be compared with those of hepatectomized, and of eviscerated rabbits that remain in good shape and that have practically a constant utilization throughout (1, 8).

It appeared to us that much of the trouble experienced by previous workers resulted from their use of dogs. We decided to substitute rabbits for this work. This animal becomes diabetic after pancreatectomy (9), and one is able to study such animals after evisceration, i.e., removal of the liver and intestinal tract. They withstand this procedure remarkably well. They make good recovery from operation, sit up normally, the kidneys excrete urine and they survive for many hours, and so satisfy the requirements which we have demanded in this laboratory for the glucose utilization rate determination after hepatectomy or evisceration.

METHOD. Pancreatectomy was performed by the method of Greeley (9) and at this time a silk ligature was placed around the inferior vena cava just above the right renal vein which constricted this vein to approximately 1.8 mm. This was done as a necessary preliminary preparation for eviscer-

TABLE 1

NUM-BER	SEX	WEIGHT AFTER EVISCERA-TION	DURATION OF DIABETES	BLOOD SUGAR BEFORE EVISCER-ATION	GLYCO-SURIA AFTER EVISCERA-TION FOR 6 HOUR PERIOD	UTILIZA-TION OF GLUCOSE PER KILO HOUR AFTER EVISCERA-TION (COR-RECTED FOR GLY-COSURIA)	O₂ UTILI-ZATION PER KILO PER HOUR	PREOPERATIVE NUTRITIONAL STATE
		grams	days	mgm. per cent	grams	grams	cc.	
1	F	1350	7	380	0.042	0.217	520	Fed
2	M	1075	4	320	0.152	0.201	351	Fed
3	M	1225	4	322	0.252	0.149	440	Fed
4	M	1220	9	380	0.018	0.053	216	Fed
5	F	1300	8	400	0.222	0.115	540	Fasted 2 days

ation (8). About two weeks later the remaining pancreas in the duodenal loop was removed. Complete evisceration, except for the urogenital system, was performed from 3 to 22 days after pancreatectomy.

As shown in table 1 some animals were fed up to the time of the evisceration and others were fasted for two or three days. Evisceration was done under ether anesthesia, the bladder washed and a catheter left in place in order to measure the glucose excreted during any given period. The blood sugar found immediately before evisceration was maintained after evisceration by the intermittent intravenous injection of glucose every thirty or sixty minutes. Glucose utilization was determined by subtracting the amount of sugar lost in the urine from the glucose administered intravenously to maintain the constant blood sugar level. Oxygen consumption was determined by connecting the tracheal cannula, which had been inserted at the time of evisceration, to a closed circuit

which contained a soda lime cartridge for the removal of CO_2, and a pump
to insure circulation of air, and a small Krogh spirometer to register the
oxygen used. The temperature of the animal was maintained during
evisceration by an ordinary electric heating pad. Kidney function was
tested by the intravenous injection of 1 cc. of 0.1 per cent phenol red as
previously described (5). Eleven animals were carried out in this way.

RESULTS. Any animals that did not appear absolutely normal after
operation or did not have functioning kidneys were eliminated. The
results are given in table 1 for animals that satisfied all requirements and
in whom the glucose utilization rate was constant during the period of

TABLE 2

NUM-BER	SEX	WEIGHT AFTER EVISCER-ATION	DURA-TION OF DIA-BETES	BLOOD SUGAR BEFORE EVISCER-ATION	UTILIZA-TION OF GLUCOSE PER KILO PER HOUR AFTER EVIS-CERATION (CORRECTED FOR GLY-COSURIA)	GLYCO-SURIA AFTER EVISCER-ATION FOR 6 HOUR PERIOD	O₂ UTILI-ZATION PER KILO PER HOUR	PREOPERATIVE NUTRITIONAL STATE
		grams	days	mgm. per cent	grams	grams	cc.	
1	F	1150	9	500	0.224* 0.324†	0.041	422	Fed
2		1370	7	110	0.109* 0.360†	0.00	354	Fasted 2 days non-fasting blood sugar 331
3	M	1175	22	204	0.172* 0.383†	0.00	413	Fasted 3 days non-fasting blood sugar 364
4	M	1675	13	160	0.119* 0.348†	0.00	358	Fasted 3 days non-fasting blood sugar 470
5	M	1150	16	100	0.174* 0.348†	0.00	234	Fasted 2 days non-fasting blood sugar 400

* First 3 hours.
† Last 3 hours.

determination. There was a group of animals that satisfied the require-
ments above but in which the rate of glucose utilization increased pro-
gressively the longer the determination was carried. We have noted this
type of behavior before in eviscerated rabbits (5) although it is not usual.
Ordinarily the rate does not change significantly. These animals are given
separately (table 2) as it appears to us that it would be a mistake to report
the average figure for the glucose utilization rate when this has changed
during the run. For these animals therefore we give both the rate for the
first 3 hours of the run and that for the later period when it was much
higher. It seems most likely that some unknown factor begins to operate
as time goes on in these animals to increase their rates and that the rate
found soon after operation is closest to the glucose utilization of the tissues
before operation.

DISCUSSION. We have not made muscle glycogen determinations in order to correct our utilization rates for the changes that might occur in muscle glycogen, as is done by Soskin and Levine (10). In hepatectomized animals the muscle glycogen practically always stays level or goes down. In doing glucose balance studies, if there is a rise in muscle glycogen it is proper that one should correct for the increase since it would probably have come from glucose. But it does not follow that one must make a similar correction for a drop in muscle glycogen when the liver is absent, for in such preparations there is no way for conversion of muscle glycogen to utilizable glucose. When muscle glycogen disappears from such animals it does not go to "spare" the glucose that has to be injected to keep the blood sugar constant; it seems chiefly to be changed to lactic acid which either accumulates in the body or is burned as such.

Before anything else we would want to stress the need of caution in interpretation of results obtained from hepatectomized diabetic animals. Such animals sometimes show large changes in glucose utilization and one must guard against making too hasty conclusions from calculations based on such results. · Young (11) on the figures from one such animal attempts to prove that fat must be changed to carbohydrate. If one wished to select certain results in our series one could make similar conclusions. The opposite could easily be proven with another selection.

Our results support the contention that the tissues of the fasting diabetic animal use glucose at the same rate as the normal. Indeed the results of some of our animals would indicate that the rate is higher in the diabetics, but there is the objection to these animals that their rates were not steady after operation, but increased after a time. No animal having a steady rate showed a higher rate than normal animals. If we accept the view that the rates obtained during the first 3 hours after operation are the closest to the rates of sugar utilization by the tissues before operation, we obtain an average figure of 138 mgm. per kilo per hour. To supply such an amount of glucose to the tissues would not necessitate a conversion of fat to glucose. The fasting rabbit excretes about 25 mgm. of nitrogen per kilo per hour and if we accept a D:N ratio of 6 (12) this amount of glucose could be supplied by the conversion of the protein to glucose.

We confirm the findings of Mann (6) and of Yater et al. (4) that diabetic tissues can and do utilize glucose. In addition we can conclude that quantitatively the tissues of the fasted eviscerated diabetic rabbit utilize glucose at the same rate as the normal fasted hepatectomized rabbit which is 125 mgm. per kilo per hour (13). The simple hepatectomized rabbit has his pancreas intact and there is no reason to suppose that it is not functioning in a normal manner. We may conclude then that the presence or absence of the pancreas has no effect on the rate of oxidation of glucose by the tissues of the fasting animal. Our results do not bear on the problem

of glucose utilization as a source of muscle energy in the normal and diabetic states. Muscle activity in our animals was quite low. It might well be that there could be a difference in the glucose utilization of diabetics and normal animals after hepatectomy, if there were a high turnover of muscle energy. Such a difference has been suggested by Yater et al. (4), based on their own findings and those of Cruikshank (14) and of Knowlton and Starling (15). In our work we may be only comparing utilizations of "basal" tissues like the brain, heart and resting muscle.

One cannot determine the glucose utilization rate of the fed hepatectomized rabbit since here there would be an undetermined absorption of glucose from the intestine. When we compare the fed normal and the fed diabetic rabbit after evisceration, we find again that quantitatively the tissue utilization of glucose is the same. We have found an average of 198 mgm. per kilo per hour for the eviscerated fed diabetic rabbit (after eliminating rabbit 4 in table 1 on account of the low oxygen utilization). Bergman and Drury have found that the eviscerated fed rabbit used glucose at the rate of 206 mgm. per kilo per hour (5). They also showed that the increase in rate over fasted eviscerated rabbits (110 mgm. per kilo per hour) was not connected with any insulin mechanism. However, even with this increase the glucose utilization is not enough to take care of the entire needs of the body. We have obtained an average oxygen consumption of eviscerated rabbits of 385 cc. per kilo per hour; such an amount of oxygen could burn 470 mgm. of glucose. If then in the fed state, an animal can obtain all his energy from the burning of glucose he cannot do so entirely by mechanisms present in the eviscerated animal. There must be other mechanisms in the "non-eviscerated" or intact animal which play a rôle in the burning of glucose. It is possible that the pancreatic function is such a mechanism and that in the fed intact animal insulin increases the oxidation of sugar and that the difference noted above may be taken care of by a burning of glucose mediated by insulin.

SUMMARY

Evidence is presented for caution in drawing too hasty conclusions as to the glucose utilization rates of hepatectomized or eviscerated animals; particular care must be exercised in case the animal is already diabetic.

Our results indicate that under basal conditions the glucose utilization rate of diabetic tissues is the same as that of normal.

The amount of glucose needed to keep diabetic eviscerated animals in a steady state accounts for between a sixth and a third of the energy requirements of the animals. The same is the case for normals after evisceration.

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INFLUENCE OF CARBON DIOXIDE ON THE EXCITABILITY OF THE VASOMOTOR CENTER IN HYPOGLYCEMIA[1]

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In an earlier investigation, Gellhorn in collaboration with Ingraham and Moldavsky (1938–39) and Kraines (1939) showed that in hypoglycemia, the blood pressure response to anoxia is markedly increased during the hypoglycemic state. Similarly it was found (Yesinick and Gellhorn, 1939) that anoxia as well as hypoglycemia increases the blood pressure response to a standardized raised intracranial pressure. Provided that the period of anoxia is not too long, anoxia may show a greater blood sugar raising effect during hypoglycemia than is observed at normal blood sugar levels (Gellhorn and Packer, 1939–40). All these effects could be understood on the basis that anoxia as well as hypoglycemia diminishes the rate of oxidations in the central nervous system. Therefore, the two factors combined would have a greater effect than each one separately. The question arose as to whether during the state of hypoglycemia the sympathetic centers are in a state of increased excitability which results in increased reactivity even to other factors which do not influence the metabolism of the brain as profoundly as anoxia. For this reason, experiments were performed on the interaction of carbon dioxide and hypoglycemia relative to the blood pressure in normal anesthetized dogs and in animals with the buffer nerves removed.

METHOD. The experiments were performed on 14 dogs anesthetized with 55 mgm./kgm. sodium amytal intraperitoneally. Artificial respiration was instituted, the femoral vein was cannulated and the blood pressure was recorded from the carotid artery. In the "denervated" dogs both vagi were cut and the carotid sinuses were removed bilaterally. The success of the complete denervation was tested by the blood pressure reaction to gas mixtures low in oxygen (Gellhorn and Lambert). The dogs inhaled CO_2-air mixtures from Douglas bags which varied in concentrations between 5 and 15 per cent for periods of 1 to 2 minutes. Insulin (Lilly)[2] was used and 3 units/kgm. were given intravenously and the same quantity intramuscularly. The blood sugar was determined by the

Somogyi modification of the Shaffer-Hartman method.   The protein was precipitated by zinc sulfate and barium hydroxide.

RESULTS.   A typical result (fig. 1) shows that with falling blood sugar, the blood pressure rises considerably more in response to carbon dioxide than it does at normal blood sugar level.   When low concentrations (about 5 per cent) of CO_2 are used, it is frequently seen that at normal blood sugar level, the blood pressure remains unchanged, but shows a considerable rise when the blood sugar has fallen to 40 mgm. per cent or below.   When
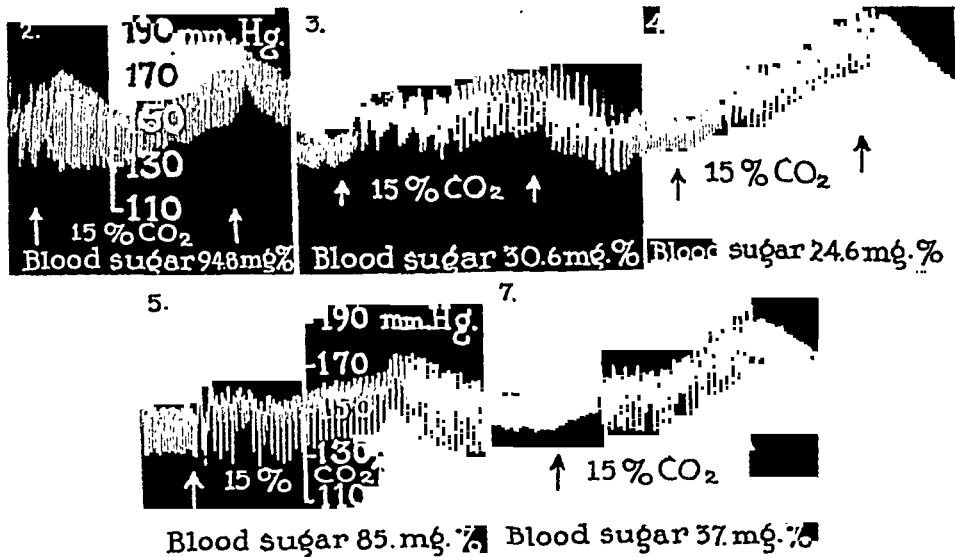
Fig. 1. 4/13/39 Female dog.   8.1 kgm.   Sodium amytal 5.5 per cent intraperitoneally 1 cc/kilo.   Artificial respiration.   The gas mixture is inhaled for 2 minutes (between the two arrows).

2, 15 per cent CO_2.   Blood sugar 94.8 mgm. per cent.   Between 2 and 3, three units of insulin per kilo are injected intravenously and three units per kilo intramuscularly.

3, 15 per cent CO_2.   Blood sugar 30.6 mgm. per cent.

4, 15 per cent CO_2.   Blood sugar 24.6 mgm. per cent.   Between 4 and 5, 50 cc. 10 per cent glucose were injected intravenously.

5, 15 per cent CO_2.   Blood sugar 85 mgm. per cent.

7, 15 per cent CO_2.   Blood sugar 37 mgm. per cent.

higher concentrations of CO_2 (15 per cent) are used, the blood pressure response at normal sugar level is somewhat variable in different animals. Sometimes it is found that the blood pressure shows a moderate fall.   In other cases (fig. 1) the blood pressure rises after a slight initial fall.   Figure 1 shows that when the blood sugar falls to 30.6 mgm. per cent, the blood pressure rises immediately in response to 15 per cent CO_2 and this rise is greatly increased when the blood sugar falls to a still lower level (24.6 mgm. per cent).   The reaction is quite reversible as shown by the further

course of the experiment illustrated in figure 1. Not infrequently, the blood sugar will fall again due to the continued action of insulin after it has temporarily been restored to approximately normal levels by the injection of glucose. If that is the case, the increased rise of blood pressure in response to carbon dioxide may occur again.

A closer scrutiny of figure 1 shows that whereas in the control experiment at the beginning (no. 2) the inhalation of CO_2 alters the blood pressure level without revealing any changes in heart action, the heart action becomes irregular and marked vagal pulses appear as the blood sugar falls. This seems to indicate that hypoglycemia sensitizes not only the vasomotor but also the cardioinhibitory center to carbon dioxide[3] but it is interesting to note that in spite of marked vagal stimulation, the total effect on the blood pressure is that of increased sympathetic and not parasympathetic activity. Apparently hypoglycemia sensitizes the parasympathetic system to CO_2 to such a degree that even after restoration of the blood sugar to 85 mgm. per cent vagal pulses occur in response to carbon dioxide although the sympathetic excitability has been restored to a normal level.

In another series of experiments, the effect of carbon dioxide inhalation on the blood pressure during hypoglycemia was studied in animals in which the buffer nerves had been removed. Figure 2 shows a typical example. At 1, the dog inhaled 7.1 per cent O_2 for 2 minutes and showed a fall in blood pressure which is characteristic of the "denervated" animal (Gellhorn and Lambert, 1939). Then 5.4 and 15 per cent CO_2 were inhaled for 2 and 1 minutes respectively. In both cases, the blood pressure fell but with the low concentration of carbon dioxide, the alteration in blood pressure was very slight. When, after injection of insulin, the blood pressure had fallen to 21 mgm. per cent, the lower concentration of carbon dioxide caused a very marked rise in blood pressure but even with 15 per cent CO_2 a rise in blood pressure was obtained. On injection of glucose, the reactions were completely reversible.

The experiments show clearly that under conditions of hypoglycemia, the vasomotor center is sensitized to carbon dioxide in a similar way as has been found previously with regard to anoxia. In the studies on anoxia, this sensitizing action referred not to a direct interaction of anoxia and hypoglycemia on the vasomotor center, but rather to an increased response of the vasomotor center to the excitatory impulses originating in the chemoreceptors of the carotid sinus and the arch of the aorta. Since in the present experiments, the sensitization holds true for both the normal and the "denervated" animal, it may be said that the vasomotor center itself is sensitized to carbon dioxide by hypoglycemia. The occurrence of vagal impulses during the inhalation of CO_2 in the hypoglycemic animal

[3] Heymans, Bouckaert and Samaan (1933) report that carbon dioxide acts directly on the vagal center.

indicates that not only the sympathetic but also the vagal center is sensitized to CO_2 but it is characteristic that the normal preponderance of the sympathetic over the parasympathetic is not only maintained but greatly increased in hypoglycemia as the blood pressure response indicates (cf. Domm and Gellhorn). As to the fact that a depressor response to high concentrations of carbon dioxide is changed into a pressor response in hypoglycemia, the following explanation seems probable. The blood pressure effect of carbon dioxide is the resultant of its peripheral action on
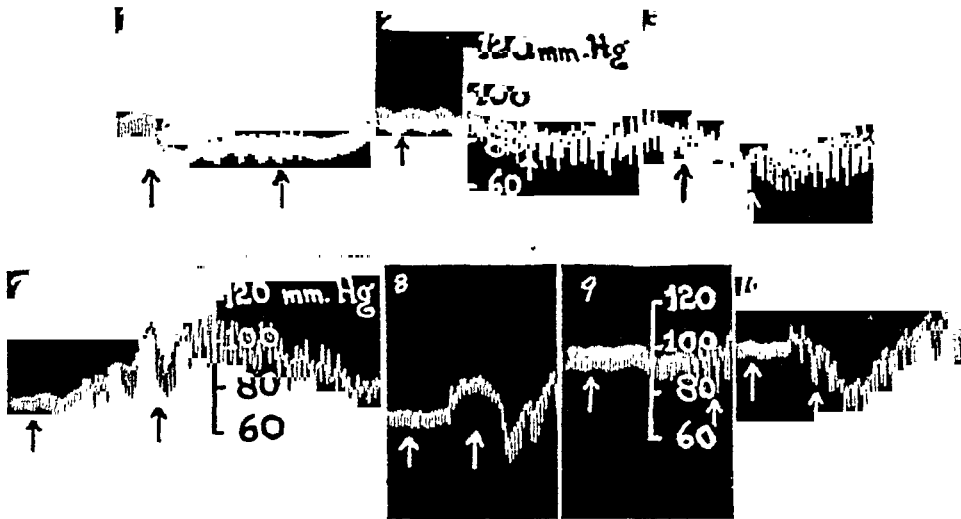
Fig. 2. 5/11/39 Male dog. 8 kgm. Sodium amytal 5.5 per cent intraperitoneally 1 cc/kgm. Both carotid sinuses denervated and vagi cut. Artificial respiration; 15 per cent CO_2 and 5.4 per cent CO_2 are inhaled for 1 and 2 minutes respectively

1, 7.1 per cent O_2 for 2 minutes (between the arrows).

2, 5.4 per cent CO_2. Blood sugar 69 mgm. per cent during 2 and 3.

3, 15 per cent CO_2. Blood sugar 69 mgm. per cent during 2 and 3. Between 3 and 4, three units of insulin per kilo are injected intravenously and three units per kilo intramuscularly.

7, 5.4 per cent CO_2. Blood sugar 21 mgm. per cent during 7 and 8.

8, 15 per cent CO_2. Blood sugar 21 mgm. per cent during 7 and 8. 50 cc. 10 per cent glucose intravenously between 8 and 9.

9, 5.4 CO_2. Blood sugar 75.5 mgm. per cent during 9 and 10.

10, 15 per cent CO_2. Blood sugar 75.5 mgm. per cent during 9 and 10.

the blood vessels (vasodilatation) and its stimulation of the vasomotor center. If the former is strong enough, a fall in blood pressure may supervene. In such a case the greatly increased response of the vasomotor center to carbon dioxide which occurs in hypoglycemia may be sufficient to counteract the peripheral effects of CO_2 and result in a marked pressor response. The situation is similar to that observed by Lambert and Gellhorn in their studies on the influence of various depths of narcosis on the blood pressure effect of carbon dioxide.

SUMMARY AND CONCLUSIONS

Experiments are reported in which in anesthetized normal dogs with and without buffer nerves, the blood pressure response to various concentrations of carbon dioxide was determined at various blood sugar levels. It was found that the blood pressure rise to CO_2 increases with falling blood sugar. If, as is frequently the case when high concentrations (15 per cent) of carbon dioxide are used, the response to CO_2 at a normal blood sugar level consists in a fall in blood pressure, this reaction may be converted into a rise when the blood sugar falls below 40 mgm. per cent. The reaction is completely reversible on injection of glucose.

It is seen not infrequently that at a low blood sugar level, vagal pulses appear in response to carbon dioxide indicating that sympathetic and parasympathetic centers are sensitized to carbon dioxide by the hypoglycemic state. However, the physiological predominance of the sympathetic over the parasympathetic is not only maintained but increased in hypoglycemia.

Addendum. Van Harreveld and McRary (Proc. Soc. Exp. Biol. Med., **43**: 564, 1940) interpret our earlier work on the interaction of anoxia and hypoglycemia as due to a diminution of the buffer reflexes rather than to an increased excitability of medullary centers. The experiments described in this paper which show an increased response to CO_2 even in the "denervated" animal clearly disprove this interpretation. Moreover, our interpretation is supported by Yesinick and Gellhorn (this JOURNAL **128**: 185, 1939) who found that increased intracranial pressure produces a greater rise in blood pressure during hypoglycemia than at normal blood sugar and that this effect persists in the absence of the buffer nerves.

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STUDIES ON HYPOGLYCEMIC AND ANOXIC CONVULSIONS[1]

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In 1939 McQuarrie and Ziegler published investigations in which they studied the effect of prolonged anoxia on the sensitivity of dogs to insulin convulsions. They observed that whereas dogs not subjected to anoxia showed regularly insulin convulsions, these were absent when the animals were subjected to anoxia of varying degrees of severity for several hours. Furthermore, McQuarrie and Ziegler established the important fact that convulsions were absent although the blood sugar level in the dog subjected to anoxia and hypoglycemia was lower than in the dog treated with insulin alone. The experiments were conducted in a similar manner as those by Glickman and Gellhorn (1938) the main difference being that these authors used rats instead of dogs and produced anoxia, not by the inhalation of suitable oxygen-nitrogen mixtures, but by reducing the barometric pressure. Nevertheless the results were apparently in contradiction to those observed by McQuarrie and Ziegler since in rats subjected to insulin and lowered barometric pressure convulsions occurred earlier and showed a much greater fatality rate than was observed in animals subjected to insulin alone. In order to explain this apparent discrepancy, experiments were carried out in which the interaction of anoxia and hypoglycemia involving convulsive insulin concentrations was studied in two different species. For one, the rabbit was chosen because numerous experiments proved that the unanesthetized rabbit lends itself particularly well to repeated studies on insulin hypoglycemia on account of the constancy of the hypoglycemic reaction. The other experiments were carried out in rats in a manner similar to that used in the work of Glickman and Gellhorn with the modification that now oxygen-nitrogen mixtures were employed instead of the low pressure chamber. In other words, conditions in the experiments performed on rats and rabbits were practically identical. Therefore, this procedure should allow one to decide whether the discrepancy in the results of Glickman and Gellhorn and those of McQuarrie and Ziegler is due to species differences or to other factors.

METHODS. All animals were starved for eighteen hours prior to experiment. Insulin (Eli Lilly & Co.)[2] was injected subcutaneously in rabbits (2.5 units/kgm.) and intraperitoneally (10 units/kgm.) in rats. Oxygen-nitrogen mixtures containing 7 per cent oxygen were made by the use of calibrated flow meters. They were administered to the rabbits by means of a metal tube which served as a head holder and was provided on one side with a perforated rubber dam sealing the mouth airtight. The gas mixtures were inhaled from Douglas bags through two valves. The rats were placed in airtight glass bottles, through which the gas

TABLE 1

MINUTES AFTER INJECTION OF INSULIN				OCCURRENCE OF CONVULSIONS
0	60	90	120	
(A) The effect of 2.5 units of insulin per kgm. on the blood sugar of rabbits				
82.7	30.6	27.8		At 106 minutes
81.1	32.2	30.6		At 103 minutes
78.5	38.7	30.6		At 102 minutes
91.3	32.2	27.8		At 91 minutes
93.5	24.1	22.2		At 115 minutes
72.0	28.5			At 83 minutes
(B) The effect of 2.5 units of insulin per kgm. on the blood sugar of rabbits subjected to 7 per cent oxygen*				
81.1	29.7	25.0	23.2	None
78.5	27.8	24.1	26.0	None
88.2	33.3	24.1	24.1	None
80.6	25.0	27.8	29.7	None
76.3	24.1	25.0	22.2	None
96.8	39.7	20.5	15.3	None

* The inhalation of 7 per cent O_2 was begun immediately after the injection of insulin and was continued for 2 hours.

mixture was drawn by suction at a speed several times greater than that corresponding to their minute volume of respiration. The gas mixtures were administered for two hours immediately after the injection of insulin. No anesthetic was used.

RESULTS. Our experiments show, first, that the administration of anoxia for two hours prevented the occurrence of convulsions in rabbits subjected to convulsive doses of insulin. Secondly, they show that in spite of this fact, the blood sugar values observed were even slightly lower in the insulin-anoxia experiments than in the insulin controls. The minimum blood sugar value observed in six anoxia-insulin experiments was

[2] Kindly supplied by Eli Lilly & Co.

23.4 mgm. per cent whereas it was 27.9 mgm. per cent in six control experiments carried out without anoxia.[3] This confirms McQuarrie and Ziegler's work and extends it to the rabbit.

McQuarrie and Ziegler had observed also that it is possible in dogs to prevent insulin convulsions by the inhalation of 15 per cent CO_2 in air, whereas convulsions regularly occur when the animal inhaled 15 per cent CO_2 in oxygen. This observation implies that when the animal inhaled 15 per cent CO_2 for a long time, some condition of anoxia evolves. Table 2 shows ten experiments in which rabbits were subjected to two hours of 15 per cent CO_2 after 2.5 U insulin per kilo were injected. In eight out of ten experiments the respiratory volumes were also determined at vari-

TABLE 2

The effect of 2.5 units insulin per kgm. on the blood sugar of rabbits which inhaled 15 per cent CO_2 for 2 hours after the injection

BLOOD SUGAR IN MGM. %, MINUTES AFTER INJECTION				RESPIRATORY VOLUME (L/MIN.) DURING INTERVAL				CONVULSIONS
0	60	90	120	0	0–30	60–90	90–120	
84.9	50.5	24.1	22.2					None
78.5	47.3	40.8	38.7					None
72.0	41.9	32.2	22.2	1.20	0.91	0.16		None
92.5	35.4	20.4	14.8	2.34	3.63	3.36	1.31	None
93.5	28.5	22.2		2.40	1.48	1.41	0.68	None
82.7	38.7	38.7		1.70	3.81	2.00	1.50	At 112 min.
91.3	28.5	28.5	31.3	1.91	1.35	0.49	0.28	None
136.5*	60.2	41.9	35.4	1.26	0.24	0.41	0.50	None
79.5	27.8	22.2	24.1	2.37	3.20	3.17	3.24	None
83.8	31.3	24.1	25.0	0.99	3.36	2.86	2.19	None

* (Struggle!)

ous intervals. It is seen that only one out of ten animals showed convulsions. Furthermore, in most of them, although not in all, the blood sugar reached convulsive levels. (Compare these values with the blood sugar values obtained in the 2.5 U/kgm. insulin control experiments shown in table 1.) In four out of eight experiments, it is seen that the respiratory volume greatly decreases during the course of the experiment, indicating that in these cases a very marked anoxia is produced. This, however, does not apply to the other four experiments in which the respiratory volume is either not reduced or is even increased. Since in three out of four experiments, convulsions are prevented in spite of adequate respiration, it seems to follow that CO_2 has some independent effect in preventing insulin convulsions. This has been confirmed in regard to other con-

[3] Compare also Gellhorn and Packer (1940).

vulsive drugs in cats in experiments which will be reported elsewhere (Yesinick and Gellhorn).

Experiments in rats showed that although the rats subjected to 7 per cent oxygen for two hours did not show any untoward effects, it was found that when they were subjected to 7 per cent oxygen plus insulin convulsions started much earlier and occurred more frequently than when insulin alone was administered. Table 3 shows that eight out of twelve rats showed hypoglycemic convulsions when injected with 10 units of insulin/kgm. and that ten out of twelve rats convulsed when injected with the same amount of insulin and subjected to 7 per cent oxygen at the same time. The latent period varied between 141 and 228 minutes in the first and 22 and 107 minutes in the latter group. The experiments confirm the work of Glickman and Gellhorn but are more striking than the results of these authors since in our new experiments no overlap exists in the latent periods of convulsions in the two groups. There is not only a quantitative but also a qualitative confirmation of the earlier work of Glickman and Gellhorn. It was mentioned in our earlier work that the type of convulsions which rats showed after insulin plus anoxia, the latter induced by the lowering of the barometric pressure, was quite different from that observed in rats subjected to insulin alone. It resembled more anoxic convulsions which may be seen either by employing oxygen-nitrogen mixtures very low in oxygen or by reducing markedly the barometric pressure. Glickman and Gellhorn came to the conclusion that "It appears as if the insulin injected animals were suffering from a relative anoxia which is greatly aggravated when the animals are exposed to a reduced oxygen pressure, although the latter is entirely harmless to non-insulinized control animals." These observations are fully confirmed in the present set of experiments. We have other indications of the essential anoxic nature of the convulsions in the animals treated with insulin and 7 per cent oxygen. Rats injected with insulin alone show convulsions that last for several minutes consisting of vigorous clonic-tonic discharges. These were almost immediately relieved by intraperitoneal injection of glucose. In sharp contradistinction to this phenomenon, it is found that rats subjected to anoxia plus insulin show very brief clonic convulsions lasting only a few seconds. In these cases the injection of glucose is of no benefit. The animals frequently die even before glucose can be injected. The anoxic nature of the convulsions observed in rats is substantiated by the third group of experiments reported in table 3. Ten rats were again subjected to insulin and 7 per cent oxygen and early convulsions of the anoxic type occurred in all animals. As soon as they appeared, 100 per cent oxygen was administered and continued for about twenty minutes. Seven out of ten rats recovered whereas none of the animals recuperated when injected with glucose (group II of table 3).

TABLE 3

I. Effect of 10 units insulin/kgm. (intraperitoneally) on normal rats

NUMBER	LATENT PERIOD OF HYPO-GLYCEMIC CONVULSIONS	REMARKS
	minutes	
1	223	
2	228	} Recover on injection of glucose
3	∞	
4	∞	
5	152	
6	217	} Recover on injection of glucose
7	188	
8	∞	
9	159	
10	141	} Recover on injection of glucose
11	153	
12	∞	

II. Effect of 10 units insulin/kgm. (intraperitoneally) on rats subjected for 2 hours to inhalation of 7 per cent oxygen

NUMBER	LATENT PERIOD OF CON-VULSIONS (ANOXIC TYPE)	REMARKS
	minutes	
1	28	
2	51	} All died in spite of injection of glucose
3	107	
4	∞	
5	37	
6	48	} All died in spite of injection of glucose
7	63	
8	∞	
9	27	
10	22	} All died in spite of injection of glucose
11	23	
12	61	

III. Effect of 10 units insulin/kgm. (intraperitoneally) on rats subjected for 2 hours to inhalation of 7 per cent oxygen

NUMBER	LATENT PERIOD OF CONVULSIONS (ANOXIC TYPE)	TREATED IMMEDIATELY WITH OXYGEN	SHOW HYPOGLYCEMIC CONVULSIONS AFTER
	minutes		*minutes*
1	47	Recover	179*
2	46	Died	
3	31	Recover	
4	58	Recover	
5	46	Recover	
6	26	Recover	190*
7	30	Recover	143*
8	51	Died	
9	60	Died	
10	94	Recover	

* All recover on injection of glucose.

Moreover, it was found that some of the rats thus saved from the otherwise fatal effect of anoxia during insulin hypoglycemia, showed two to three hours later hypoglycemic convulsions which could be promptly alleviated by injection of glucose.

We therefore come to the conclusion that the rabbits in our experiments behaved similarly to the dogs in the experiments of McQuarrie and Ziegler. In these animals, insulin convulsions are prevented by anoxia. In the rats, however, no matter whether a low barometric pressure or the inhalation of oxygen-nitrogen mixtures is used for inducing anoxia, the animals subjected to insulin plus anoxia die under convulsions, whereas convulsions occur only later or not at all in the animals treated with insulin alone. But it seems to be unfair to conclude from these experiments that insulin convulsions are prevented by anoxia in rabbits and dogs but are precipitated by anoxia in rats. In confirmation of the earlier work of Glickman and Gellhorn, we found in our new experiments on rats that the convulsions observed in hypoglycemic and anoxic animals are definitely of an anoxic and not of a hypoglycemic nature. We may, therefore, say that in all animals thus far studied with regard to the interaction of anoxia and hypoglycemia, anoxia prevents insulin convulsions, but in the rats, anoxic convulsions occur which are not seen either in the dog or in the rabbit subjected to similar procedures. If we take into consideration the fact that both hypoglycemia and anoxia diminish the rate of oxidations in the brain, the difference between rats on one side and rabbits and dogs on the other side seems to be not a difference in sensitivity to hypoglycemic convulsions but a difference in sensitivity to oxygen deficiency. In the case of rats, anoxia when superimposed on hypoglycemia, creates such a diminution of oxidative processes in the brain that these animals show anoxic convulsions. Rabbits in general do not show these symptoms, although in a few cases with denervated adrenals we saw a similar behavior and then the injection of glucose was of no avail. The conclusion which we arrive at that a similar diminution in the oxidation rate of the brain may produce more marked anoxic symptoms in rats than in rabbits and dogs, is supported by the experience of Haldane that small animals such as mice and birds are more sensitive to carbon monoxide than are larger animals, including man. It is this specific sensitivity to anoxia, characteristic of small animals with a high metabolic rate, which accounts for the differences observed in the two groups of experiments. The conclusion seems imperative that insulin convulsions require a higher degree of oxygenation of the brain centers than is present when the animals are subjected to anoxia during the hypoglycemic state. The occurrence of anoxic convulsions in rats subjected to insulin plus 7 per cent oxygen furnishes a further illustration of the synergistic action of anoxia and hypoglycemia on the central nervous system (cf. Gellhorn and collaborators, 1938–1940).

SUMMARY

1. Rabbits injected with 2.5 units of insulin subcutaneously and subjected to 7 per cent oxygen for two hours do not show insulin convulsions in spite of convulsive blood sugar levels. This confirms the work of McQuarrie and Ziegler. If, instead of low oxygen, 15 per cent CO_2 is inhaled, the results are similar to the experiments with anoxia. In 50 per cent of the rabbits, marked signs of respiratory failure are observed indicating that under these conditions, a marked diminution in the oxidative processes takes place also.

2. In experiments performed on rats which were given 10 units of insulin/kgm. intraperitoneally and subjected to 7 per cent oxygen for two hours, the animals show convulsions much earlier than the control animals subjected to insulin alone, but the nature of these convulsions is anoxic and clinically different from insulin convulsions. This is confirmed by the fact that whereas injection of glucose immediately restores the animals suffering from insulin convulsions, it has no beneficial effect on animals showing anoxic convulsions. Moreover, such convulsions can be alleviated by administration of pure oxygen. The difference in the behavior of rats and rabbits is explained on the basis of the greater oxygen sensitivity of small animals showing a high metabolic rate. It is concluded that in all species thus far studied, anoxia prevents insulin convulsions.

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ACTIONS OF ADRENALINE AND ACETYLCHOLINE ON THE DENERVATED IRIS OF THE CAT AND MONKEY

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It has been shown repeatedly that when structures supplied by sympathetic nerves are deprived of their post-ganglionic supply, they become sensitive to circulating epinephrine. Likewise, when parasympathetically innervated muscle is deprived of its post-ganglionic supply, it contracts readily when in contact with acetylcholine. Shen and Cannon (1936) found that after excision of the ciliary ganglion in the cat, the paralyzed circular fibers of the iris constricted the pupil on the instillation of 1.0 per cent acetylcholine into the conjunctival sac. This miosis was augmented by previous application of eserine. In this communication comparative studies are reported of the effects of adrenaline and acetylcholine on the sympathetically and parasympathetically denervated irises of the cat and monkey.[3]

METHOD. In the following experiments, cats and monkeys (Macaca mulatta) were used. In 60 cats and 20 monkeys, the superior cervical ganglion was removed, and in 30 cats and 7 monkeys the short and long ciliary nerves were sectioned. The latter procedure was a modification of the operation devised by Anderson (1905), and used by Shen and Cannon (1936). The method of exposure of the ciliary nerves in the monkey was similar to that used in the cat except that in the former it was necessary to trephine the lateral wall of the bony orbit.

The superior cervical ganglion was removed in the usual manner. The changes in pupillary diameter were recorded in most instances by measuring the horizontal diameter with a millimeter scale, and in some photographically.

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[3] For purposes of brevity, the iris deprived of its post-ganglionic sympathetic nerve supply will be designated as the *s.d.*, i.e., sympathetically denervated iris, while the iris of the eye in which the post-ganglionic ciliary and optic nerves were sectioned will be referred to as the *c.d.*, i.e., completely denervated iris. In the completely denervated iris, both the sympathetic and parasympathetic nerve supplies were abolished.

In order to visualize the pupil better, the nictitating membrane was removed in a number of cats. In chronic experiments, the eye was bandaged with 1:5000 bichloride of mercury ointment, so as to avoid ulcerations of the cornea.

The drugs, i.e., adrenaline hydrochloride (Parke Davis) and acetylcholine hydrochloride (Merck) were used with the animal in most instances unanesthetized. The 1.0 per cent adrenaline solution used for local application to the eye was prepared from crystalline epinephrine bitartrate (Winthrop). All dilutions were made with saline to volume of 1.0 cc. The dosages recorded in the subsequent experimental data are per kilogram of body weight, unless otherwise noted.

RESULTS. I. *Adrenaline* (a) *cat*. The normal iris and that deprived of its sympathetic innervation by preganglionic section dilated with intravenous injections of adrenaline in amounts not less than 5 gamma, while the iris in which the post-ganglionic sympathetic fibers were cut dilated with much smaller doses. Intravenous injection of adrenaline, 5 gamma or more, excited the radial fibers of the iris of the cat even immediately after their complete denervation. Following this operation, the sensitivity of the denervated pupillodilator muscle to epinephrine increased with time. The minimal dose of epinephrine necessary to dilate the *s.d.* iris was 0.08 gamma. The sensitivity of the radial fibers of the *c.d.* iris was even greater, 0.02 gamma produced a minimal dilatation. When injected into the ipsilateral common carotid artery, the quantity of epinephrine required to dilate the *c.d.* iris was 0.0005 gamma.

(b) *Monkey*. In the monkey, the sensitivity of the *s.d.* iris to intravenous injections of adrenaline was not as marked as in the cat. In only two macaques were 4.0 gamma of adrenaline effective in producing mydriasis of the *s.d.* iris. In all other instances from 50 to 200 gamma were necessary. The mydriasis was never conspicuous or maximal, the pupil increasing only by 1.0 to 2.5 mm. over the original diameter. In three monkeys, the pupil became irregular and there was hemorrhage into the anterior chamber of the eye. In monkeys with preganglionic section at the stellate or at the middle cervical ganglion, large doses of intravenously injected adrenaline sometimes enlarged the ipsilateral pupil. The normally innervated iris did not dilate with intravenous injections of adrenaline.[4]

Unlike the cat, complete denervation of the monkey's iris did not

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[4] These pupillary dilator reactions noted in the cat and monkey, following the administration of adrenaline, are in accord with the observations of the mydriasis obtained when each is frightened. Frightening the cat produces a marked dilatation of the *s.d.* and *c.d.* irises presumably due to a discharge of adrenaline. In the monkey there is no change in pupillary diameter with fright and struggle unless cocaine is given locally or parenterally (Fulton, 1940).

enhance the sensitivity to epinephrine even when large doses ranging from 200 to 1000 gamma were employed. The mydriasis was slight and frequently did not occur. Instead a progressive constriction of the pupil ensued from 45 to 90 seconds after the adrenaline was injected (fig. 1A). This delayed miosis of the c.d. iris caused by adrenaline could be enhanced by eserine, given 15 minutes previously either in the conjunctival sac or intramuscularly, and could be diminished or abolished by parenteral atropine. Eserine thus given had no visible effect on the
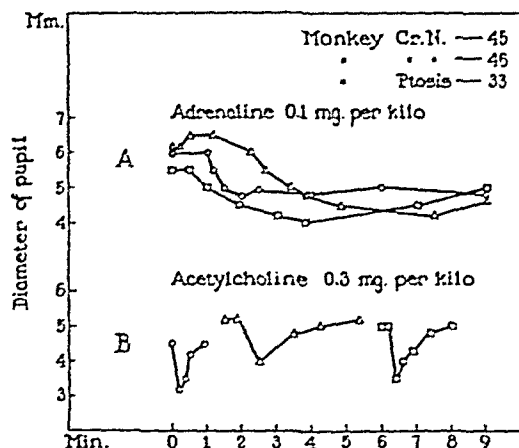
Fig. 1   Fig. 2

Fig. 1. Three unanesthetized monkeys each with c.d. iris. At A, injected with adrenaline into saphenous vein. At B, injected with acetylcholine by vein. Note the long interval between injection of epinephrine, first point indicated, and appearance of miosis following the drug injection.
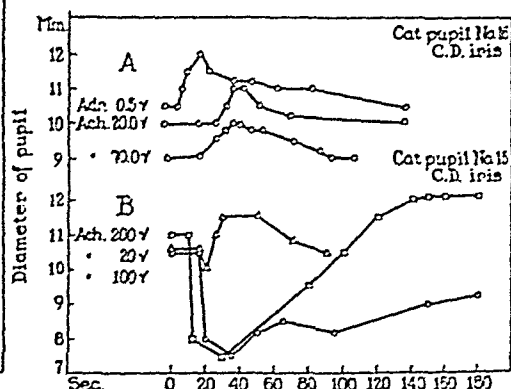
Fig. 2. Unanesthetized cat with c.d. iris. Weight 2.8 kgm. Effect of intravenous injections of acetylcholine on pupillary diameter in amounts as indicated. Pupillary constriction could be elicited immediately after the denervation.

A. Comparative actions of adrenaline and acetylcholine. Note that the adrenaline mydriasis appeared within the circulation time of 6 seconds, while acetylcholine mydriasis was delayed and appeared twenty or more seconds after the drug was injected at first point.

B. Variable constrictor and dilator effects of intravenous acetylcholine. Curve with squares represents that produced by intravenous injection of 200 gamma, with triangles 20 gamma and with circles 100 gamma of acetylcholine.

pupil. The miosis due to adrenaline could not be elicited in every monkey tested, nor on every occasion. Furthermore, the phenomenon could be demonstrated only during the period in which the denervated pupilloconstrictor muscle was hypersensitive and readily contracted with acetylcholine.

II. *Acetylcholine* (a) *cat.* In the cat, intravenous injections of acetylcholine up to 50 gamma had little effect on the normal iris. In doses greater than 6 to 10 gamma, dilatation of the s.d. iris occurred 14 to 30

seconds following the administration of the acetylcholine. This interval was considerably greater than the circulation time which is usually six seconds (fig. 2A). Eserine increased and atropine abolished or hindered the delayed mydriatic effect of acetylcholine. In most instances, bilateral adrenalectomy abolished the dilatation in the *s.d.* iris and diminished it in the normal iris. The *s.d.* and normal nictitating membranes retracted with acetylcholine even after bilateral adrenalectomy.

In the *c.d.* iris of the cat, acetylcholine administered intravenously had a dual action, mydriatic and miotic (fig. 2B). The responses varied, but the dilator effect was the more frequent. Even when miosis did occur, it preceded or followed a mydriasis. At times no change in the pupil was noted. The constrictor effect of acetylcholine on the *c.d.* iris did not seem to depend on the quantity injected, for in the same cat 10 gamma were equally effective and ineffective. Intracarotid injections of acetylcholine also yielded variable results; in one instance 4 gamma produced miosis, on other occasions there were no changes, even with as large a dose as 1 mgm. The constriction produced by acetylcholine appeared from 10 to 60 seconds after its intravenous injection into the uneserinized cat and persisted for many minutes, long after the acetylcholine must have disappeared from the circulation by the hydrolytic action of blood cholinesterase. Atropine, 1 mgm. intramuscularly, abolished the miotic and diminished the mydriatic effect of parenteral acetylcholine. With large doses of intravenously injected acetylcholine, 100 gamma or more, slight constriction of the *c.d.* iris could be elicited as early as 5 minutes after the denervation. At this early stage the usual secondary mydriasis was not observed.

(b) *Monkey.* In the monkey, intravenous injections of acetylcholine produced constriction of the *c.d.* iris (fig. 1B). The constriction was augmented and prolonged by eserine injected parenterally or applied locally to the eye. This acetylcholine miosis was also an inconstant phenomenon but there were no apparent secondary effects on the pupil, such as the delayed mydriasis observed in the cat, even when large doses of acetylcholine, 1 to 4 mgm., were administered with or without previous eserinization.

DISCUSSION. After sectioning all the ciliary sympathetic and parasympathetic nerves to the eye, both adrenergic and cholinergic sensitization phenomena may be demonstrated in the denervated iris; the pupillodilator muscle reacts with adrenaline while the pupilloconstrictor muscle reacts with acetylcholine. The actions of adrenaline and acetylcholine on the denervated iris of the cat differ from those of the monkey. The pupillary effects obtained with intravenous injections of these drugs may be determined by general and local reactions.

A. *General (reflex) reactions.* The injection of an excess amount of one

of the autonomic substances causes a reflex discharge of the other in the body. Feldberg and Minz (1931) demonstrated that acetylcholine injected into the circulation caused a secretion of epinephrine from the adrenal medulla of cats, dogs, and rabbits. Conversely, Krayer and Verney (1934) showed that when adrenaline was injected intravenously, there occurred a reflex discharge of acetylcholine into the coronary vein of the dog and cat.

It is probable that the mydriasis of the denervated iris caused by acetylcholine in the cat is in part due to a reflex discharge of epinephrine from the adrenal medulla. This is supported by the observations that bilateral adrenalectomy abolishes and in some instances diminishes the mydriasis caused by acetylcholine. The fact that bilateral adrenalectomy does not eliminate this mydriasis in every cat tested suggests that acetylcholine may release epinephrine from obscure accessory chromaffin bodies or stimulates the secretion of an adrenaline-like substance, such as sympathin, at the sympathetic nerve endings (Bacq, 1936; Cannon and Lissák, 1939). Using the s.d. nictitating membrane of the cat as an indicator, Bacq (1936) found that only after removal of the adrenals and complete sympathectomy does acetylcholine fail to retract the nictitating membrane. The mechanism of reflex discharge of epinephrine by acetylcholine may also exist in the monkey as it does in other species but the quantity so liberated may be insufficient to dilate the s.d. iris, which in this animal is found to have a low sensitivity to injected adrenaline. It is possible that if a different indicator were used, such as the denervated heart or intestine (Youmans et al., 1940), the reflexly liberated adrenaline or sympathin could be detected. The evidence and methods used to study the release of adrenaline by intravenously injected acetylcholine has been reviewed by Hermann et al., 1937.

Just as acetylcholine can stimulate the secretion of adrenaline, so may adrenaline cause the liberation of acetylcholine (Krayer and Verney, 1934). The constriction of the completely denervated iris of the monkey by intravenous injections of adrenaline may be due to a reflexly released parasympathomimetic substance. This is particularly likely since the miosis caused by adrenaline is augmented by eserine and occurs during the period the denervated pupilloconstrictor muscle is hypersensitive to acetylcholine. Additional presumptive evidence that the delayed pupillary constriction is due to a reflexly discharged chemical agent is found in experiments with denervated facial muscles of monkeys. These denervated muscles, which are extremely sensitive to acetylcholine, contract from 40 to 150 seconds after the intravenous administration of large amounts of adrenaline (Bender, 1939). The contraction is augmented by eserine, and occurs only while the denervated facial muscles are sensitive to acetylcholine. Still another example in which denervated striated muscle reacts indirectly with adrenaline are the experiments of Mahoney

and Sheehan (1936), who found that 5 days after intracranial section of the oculomotor nerve the denervated levator palpebrarum contracted *several minutes* after the intramuscular injection of 2 mgm. of epinephrine. Since the denervated ocular muscles are highly sensitive and contract readily with acetylcholine (Bender and Fulton, 1939) it is implied that the delayed contraction produced by adrenaline is due to reflex liberation of a cholinergic substance. The origin of this hypothetical, reflexly liberated cholinergic substance in the body is obscure and there is a question whether the parasympathomimetic effects are due to acetylcholine per se. Feldberg and Schriever (1936) found that intravenously injected adrenaline produced secretion of acetylcholine into the cerebrospinal fluid of eserinized dogs. According to these authors, the acetylcholine thus derived was not from the secretions of the vagal nerve endings.

The absence of the cholinergic effect following parenteral injections of adrenaline in the cat may be more apparent than real. It is possible that the dilator fibers of the iris react more powerfully than the constrictor set. Since adrenaline excites the dilator fibers, the stimulation of the constrictor set by reflexly liberated acetylcholine may not be sufficient to overcome the adrenaline mydriasis. Thus although acetylcholine may be secreted after intravenous injection of large doses of adrenaline, its presence in the cat could not be detected by the sensitized body indicators described in this report.

B. *Local (direct) reactions.* The radial and circular fibers of the iris each react with both adrenaline and acetylcholine. Cannon and Rosenblueth (1935) reported that hepatic sympathin acts on both the radial and circular fibers of the iris of the cat. Also, Rosenblueth (1932), Morrison and Acheson (1938), and Rosenblueth and Cannon (1939) found that the nictitating membrane of the cat contracts with adrenaline and acetylcholine, as we did. Similarly, it is conceivable that the constrictor and dilator sets of denervated fibers of the iris in the cat each react with acetylcholine. Since the two sets of fibers are antagonistically arranged, the final effect is the resultant of the two opposing actions. In the cat the usual response to intravenous injections of acetylcholine or adrenaline is dilator and this suggests that the dilator fibers are the more powerful of the two groups.

The same principle of a drug acting simultaneously on antagonistic sets of fibers of the iris may account for the adrenaline constriction of the *c.d.* iris of the monkey. In the monkey, however, in contrast to the cat, the pupilloconstrictor muscle may be stronger than the dilator fibers and, therefore, would respond with acetylcholine as well as with adrenaline. Although this hypothesis could explain the miosis of the *c.d.* iris it would hardly account for the phenomenon of contraction of the denervated facial striated muscles elicited by adrenaline in monkeys (Bender, 1939).

Of the two explanations offered for the pupillary responses obtained

with the autonomic drugs the experimental evidence seems to favor the theory of general hormonal reaction, namely, an excess of acetylcholine causes secretion of adrenergic substance while excess of adrenaline causes liberation of a cholinergic substance in the body. Such reflex reactions neutralize the effects of the drugs introduced into the body and thus conform with the widely established principle of homeostasis (Cannon, 1939).

The foregoing data reveal that the cat responds to autonomic drugs chiefly with the dilator, while the monkey with the constrictor mechanism of the denervated iris. Since in this case the iris is free from nervous control the changes in pupillary diameter must be due to an excitation of the iris fibers by a chemical agent either introduced or released within the body. The chemical agents liberated within the organism are presumably adrenaline and acetylcholine. These findings are similar to those noted in conditions in which the autonomic system is hyperactivated such as in fright and struggle (Bender, 1938), and in insulin-hypoglycemia (Bender and Siegal, 1940). All observations considered together suggest that both adrenergic and cholinergic like substances are elaborated in each of the species and that the cat displays predominantly the sympathomimetic, whereas the monkey shows more of the parasympathomimetic reactions to the same stimuli.

SUMMARY

1. The denervated iris of the cat and monkey differs in the reactions to the autonomic drugs. In general the cat responds with its dilator while the monkey reacts with its constrictor-fibers of the iris.

2. After sectioning of all the ciliary and optic nerves, the iris is rigid to all forms of neural stimuli but responds to chemical agents. Intravenous injections of adrenaline produce a dilatation of the c.d. iris in the cat but a delayed miosis in the monkey. Intravenous injections of acetylcholine produce an inconstant constriction and a more frequent secondary dilatation in the cat while in the monkey there is only constriction.

3. The pupillary dilatation obtained after injections of acetylcholine in the cat is usually abolished by bilateral adrenalectomy. The pupillary constriction which is noted on intravenous injections of adrenaline in the monkey is potentiated by eserinization and occurs only while the constrictor fibers of the iris are sensitive to acetylcholine. These facts suggest that excess of acetylcholine stimulates the secretion of adrenaline and excess of adrenaline stimulates the liberation of a cholinergic-like substance in the body.

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THE MECHANISM OF THE EXCRETION OF VITAMIN C BY THE DOG KIDNEY[1]

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The mechanism of the excretion of vitamin C in the human has been reported in a previous study (1, 2). It was found that in man ascorbic acid was excreted through filtration and active tubular reabsorption. The reabsorptive mechanism appeared to be limited by a maximal rate so that when the vitamin was presented to the tubules by the glomerular filtrate at a rate exceeding this maximum the excess was excreted in the urine. In a series of normals studied by us, and by Smith and his co-workers (3) it was found that the human kidney was capable of reabsorbing 1.2 to 2.1 mgm. of vitamin C per 100 cc. of glomerular filtrate with an average of 1.7 mgm. per 100 cc. of glomerular filtrate or 2.0 mgm./min. At plasma levels below saturation (2) the vitamin was almost completely reabsorbed. This reabsorption, however, was never entirely complete and some ascorbic acid appeared in the urine regardless of the plasma concentration. These data in the human appeared to uphold the hypothesis which treats tubular reabsorption as a phenomenon following the law of mass action (4). It was also shown that although in chemical structure vitamin C is related to the carbohydrates, it is not reabsorbed by the same mechanism as glucose.

Because the dog is capable of synthesizing its own vitamin C, the mechanism by which this animal excretes the vitamin was studied. In this investigation simultaneous creatinine and vitamin C clearances were done as this provides a method for studying the mechanism of the excretion of ascorbic acid in the dog.

PROCEDURE. Observations were made on four normal well-trained dogs kept on identical diets consisting of crackermeal 100 grams, skimmed milk 30 grams, yeast 10 grams, bone ash 4 grams, salt mixture 3 grams, meat powder 35 grams, cod liver oil 22 cc. The dogs were hydrated the day prior to the test and the morning of the experiment. The dogs were unanesthetized and loosely restrained upon a comfortable animal board.

Constant creatinine and varying ascorbic acid concentrations in the plasma were obtained by means of constant intravenous infusions. Urine was collected by an indwelling catheter and at the end of each period the bladder was emptied as completely as possible and washed out with saline. Bloods were obtained at frequent intervals.

Potassium oxalate was used in minimal quantities as an anticoagulant. Vitamin C was determined in plasma by the method of Mindlin and Butler (5); and in urine by the method of Evelyn et al. (6). For creatinine, the plasma and urines were precipitated using the ferric sulphate-
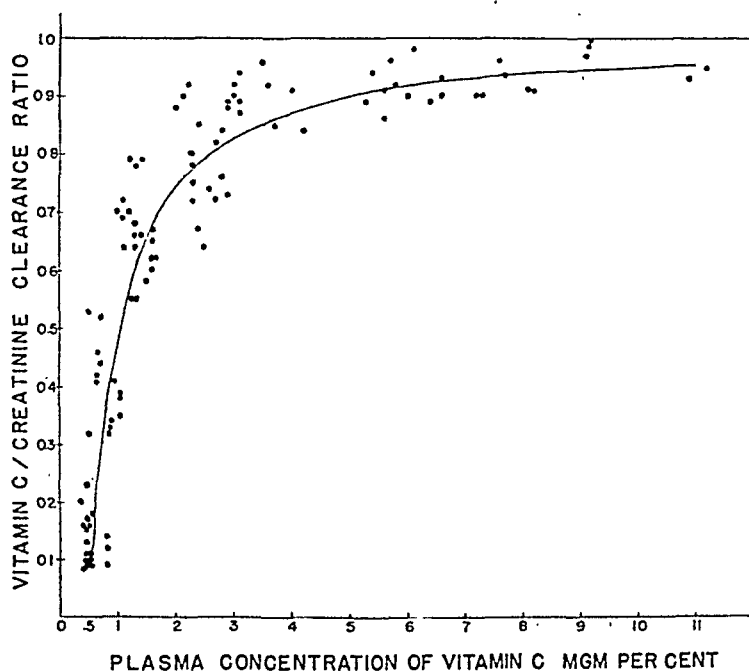
Fig. 1. The relationship between the vitamin C creatinine clearance ratio and the plasma level of vitamin C

barium carbonate method (7) and then determined by the Folin and Wu (1919) (8) method.

RESULTS. In all a total of 102 clearance periods were run on the four normal dogs. The plasma concentration of vitamin C was varied from 0.40 to 10.9 mgm. per cent. The vitamin C/creatinine clearance ratio is plotted against plasma concentration in figure 1. It should be noted that the vitamin C clearance has a low value at low plasma ascorbic acid concentrations. As the plasma concentration was raised there was a sharp rise in the vitamin C clearance which approached the creatinine clearance as a limiting value.

The data were analyzed to determine whether the renal tubules reabsorbed the vitamin up to some maximal limiting rate after which any

vitamin present in the glomerular filtrate would be excreted in the urine. The absolute amount of the vitamin reabsorbed by the tubules was calculated by subtracting the amount excreted per minute from the amount

TABLE 1

Maximal reabsorption of vitamin C

DOG' NUMBER	PLASMA CONC.	FILTERED		EXCRETED		REABSORBED	
			Glomerular filtrate		Glomerular filtrate		Glomerular filtrate
	mgm. per cent.	*mgm. per min.*	*mgm. per 100 cc.*	*mgm. per min.*	*mgm. per 100 cc.*	*mgm. per min.*	*mgm. per 100 cc.*
190	0.80	0.39	0.80	0.04	0.08	0.35	0.72
	2.36	1.24	2.36	0.86	1.64	0.38	0.72
	7.36	4.37	7.36	3.95	6.62	0.42	0.74
189	1.05	0.45	1.05	0.17	0.39	0.28	0.66
	1.62	0.83	1.62	0.51	1.00	0.32	0.62
190	0.46	0.21	0.46	0.004	0.01	0.21	0.45
	2.77	1.59	2.77	1.35	2.35	0.24	0.42
211	2.51	1.38	2.51	1.03	1.88	0.35	0.63
	6.49	4.28	6.49	3.90	5.91	0.38	0.58
190	2.77	1.64	2.77	1.21	2.03	0.43	0.74
	5.43	2.91	5.43	2.53	4.72	0.38	0.71

TABLE 2

Effect of hyperglycemia on the reabsorption of vitamin C

PLASMA VITAMIN C	PLASMA GLUCOSE	CLEARANCES			VITAMIN C CREATININE CLEARANCE RATIO	VITAMIN C REABSORBED	
		Vitamin C	Creatinine	Glucose			
mgm. per cent	*mgm. per cent*	*cc. per min.*	*cc. per min.*	*cc. per min.*		*mgm. per min.*	*mgm. per 100 cc. filtrate*
0.90	109	31.6	52.8		0.60	0.19	0.36
0.87	114	22.8	46.1		0.49	0.21	0.46
0.84	120	20.9	47.8		0.44	0.23	0.47
0.68	510	22.2	62.5	30.2	0.36	0.27	0.44
0.68	510	24.8	69.2	40.8	0.36	0.30	0.44
0.68	510	21.0	71.5	33.6	0.29	0.34	0.48

filtered per minute. Assuming that the vitamin was completely filterable from the plasma the latter value was obtained by multiplying the creatinine clearance by the plasma concentration of the vitamin.
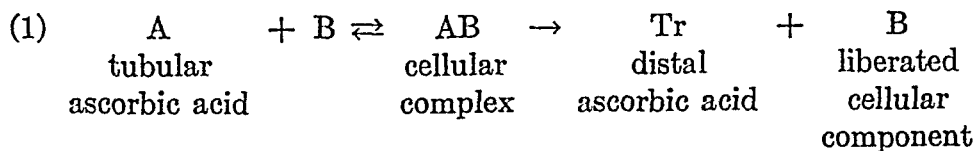
Table 1 gives the quantity of vitamin C reabsorbed by the tubules in

33 observations in 3 dogs at 10 different plasma levels, each figure being the average of 3 observations. These data indicate that there is a limitation in the capacity of the renal tubules to reabsorb the vitamin and that it is this circumstance that leads to the increased excretion at elevated plasma levels.

Another experiment was run in order to determine whether glucose and vitamin C were reabsorbed by a common mechanism in the dog. Table 2 gives the results of this test. When the plasma concentration of glucose was raised above 500 mgm. per cent, putting a maximum strain on the glucose reabsorptive system there was no impairment of the reabsorption of vitamin C. From this it may be inferred that the reabsorption of vitamin C and of glucose does not involve a common mechanism.

The data were also analyzed with a view to determining whether or not the rate of urine flow affected the excretion of vitamin C. Absolutely no relationship was found between these two when the urine flow varied from 1.0 to 8.7 cc./minute.

DISCUSSION. The validity of the calculation of the active tubular reabsorption depends on the assumption that the vitamin is completely filterable at the glomerule. Leblond (9) has demonstrated that this circumstance exists in the frog, and our work (1) on the human kidney also supports this viewpoint. The data indicate that vitamin C in the dog, as in man, is excreted only by filtration; that it is actively reabsorbed by the renal tubules, and that the factor which limits this reabsorptive process is the existence of a maximal rate. This phenomenon of a maximal rate of transfer has been shown to obtain for glucose (10) and for vitamin C in man (1) and for the tubular excretion of many organic compounds (4). Shannon (4) has suggested that this limitation may follow from the circumstance that in the process of active transfer the substance enters into reversible combination with some cellular element present in a limited and constant amount and it is the rate of decomposition of this complex which limits the progress of the over-all reaction of transfer. In the case of vitamin C this situation may be represented as follows:

$$(1) \qquad \underset{\substack{\text{tubular} \\ \text{ascorbic acid}}}{A} \quad + \underset{\substack{\text{cellular} \\ \text{complex}}}{B \rightleftarrows \quad AB} \quad \rightarrow \quad \underset{\substack{\text{distal} \\ \text{ascorbic acid}}}{Tr} \quad + \quad \underset{\substack{\text{liberated} \\ \text{cellular} \\ \text{component}}}{B}$$

Applying the law of mass action to such a condition the following relationship (4) may be derived:

$$(2) \qquad K = (a - Tr/v)\left(\frac{Tm - Tr}{Tr}\right)$$

where Tm represents the maximal rate of tubular reabsorption, v the rate of glomerular filtration in units of 100 cc., a the plasma concentration, Tr the calculated rate of tubular reabsorption and K a constant.

The smooth curve shown in figure 1 was calculated with the following equation:

(3)   Vitamin C/creatinine clearance ratio $= 1 - Tr/a$

The values for Tr were obtained by using equation 2 in which the value for Tm was 0.52 mgm./100 cc. filtrate (the average maximal rate observed in all our observations); v was equal to 100 cc./mm. and K had a value of 0.01.

The data obtained fit this calculated curve satisfactorily and indicate that the hypothesis upon which equations (1) and (2) are based can be used to describe the reabsorptive system at all plasma concentrations examined in the dog.

Therefore, in the dog, ascorbic acid is excreted by filtration and active tubular reabsorption, and the excretion of vitamin C is dependent upon the plasma concentration of the vitamin, the rate of glomerular filtration, and the maximal rate of tubular reabsorption. This mechanism of the excretion in an animal capable of synthesizing its own ascorbic acid is similar to the mechanism by which man excretes the vitamin (1). However, the ability of the tubules to reabsorb the vitamin in the dog (0.52 mgm./100 cc. filtrate) is much less than that of man, e.g., 1.7 mgm./100 cc. filtrate.

<div align="center">SUMMARY</div>

1. Simultaneous vitamin C and creatinine clearances in the normal dog show that ascorbic acid is excreted by filtration and active tubular reabsorption.

2. The reabsorptive mechanism for vitamin C appears to be limited by a maximal rate so that when the vitamin is presented to the tubules by the glomerular filtrate at a rate exceeding this maximum the excess is excreted in the urine.

3. Vitamin C is not reabsorbed by the same mechanism as glucose.

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ACETYLCHOLINE HYDROLYSIS BY THE FROG'S SARTORIUS MUSCLE[1]

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The problem of chemical transmission of motor nerve impulses to voluntary muscle has been approached by Marnay and Nachmansohn (1) by studying the choline esterolytic activity of various portions of the sartorius muscle of the European frog (Rana esculenta). Pezard and May (2) previously had shown that the pelvic one-fifth of this frog's sartorius has few motor nerve endings, while the adjacent or second fifth is richest in nerve endings and the third fifth has somewhat fewer than the second. Marnay and Nachmansohn determined the choline esterase present in these three portions and found that the second fifth has approximately seven times and the third fifth approximately four times the hydrolytic activity of the pelvic fifth. They also found that the sciatic nerve has approximately eight times the esterolytic activity shown by the pelvic fifth of the muscle and concluded that this difference in activity of the various portions of the muscle could not be attributed to the presence of nerve fibers alone, but must be evidence of a concentration of the enzyme at the nerve endings. From their data they calculate that 0.001 μg. of acetylcholine can be hydrolysed at the nerve endings of a sartorius muscle weighing 300 mgm. during the refractory period of the muscle.

Confirmation of this work comes from similar studies on the sartorius of the toad made by Feng and Ting (3) but under different experimental conditions. They found that the pelvic one-fourth has much less choline esterase than the portion of muscle near the nerve entrance.

In view of the discrepancies in experimental conditions between the work of Marnay and Nachmansohn and that of Feng and Ting, it was deemed desirable to obtain data on Rana pipiens, the frog commonly used in this country for experimental work, and some experiments are herewith reported using the procedure of Marnay and Nachmansohn.

METHOD. Sartorius muscles of Rana pipiens were removed and divided as quickly as possible into five equal portions. Those portions repre-

senting the pelvic, second, and third fifths of the muscles were weighed and ground with quartz powder and Ringer solution to form a suspension of the tissue. This suspension was transferred to volumetric flasks and made to the desired volume with Ringer solution which had the same composition as that used by Marnay and Nachmansohn. After shaking. thoroughly each time, 4 cc. portions of this suspension were transferred to Warburg vessels. The side-bulbs contained 0.5 cc. (10 mgm.) of acetylcholine bromide which will give an initial concentration of 1.6 mgm. of acetylcholine in 1 cc. The vessels were equilibrated with a 5 per cent CO_2-N_2 mixture; they were then tipped and the production of CO_2 was followed, in most cases, for 120 minutes. The temperature of the bath was 18°C.

In preliminary experiments no spontaneous hydrolysis of acetylcholine, nor spontaneous production of acid by the tissues, was found under these experimental conditions.

Previous experiments by one of us (4) have shown the necessity of prostigmine or physostigmine controls, since one sometimes gets hydrolysis of acetylcholine which is not due to choline esterase. Such controls in the experiments reported here showed that there was no hydrolysis of the ester by muscle with physostigmine present in a dilution of 1:10,000.

When working with biological fluids or tissue suspensions, buffers other than $NaHCO_3$ are present, and the production of CO_2 is not equivalent to the acetylcholine hydrolysed. This necessitates the addition of an acid retention factor to the vessel constant. Warburg, Kubowitz and Christian (5) have devised an experimental method for determining a composite flask constant (K_M) for serum by adding a known quantity (X_{CO_2}) of lactic acid to the serum in the presence of an excess of $NaHCO_3$ and measuring the CO_2 pressure (h) which is developed. The flask constant for each vessel is calculated from their equation:

$$(1) \qquad K_M = \frac{X_{CO_2}}{h}$$

Since in our work varying weights of tissue are used, K_M would not be constant from one experiment to another, and, in order to apply this method to the conditions of our experiments, it has been necessary to break the composite factor into its component parts:

$$(2) \qquad K_M = K_{CO_2}^{Ringer} + \Delta K_M$$

where ΔK_M is the retention factor for the particular weight of tissue used. From equation (2) ΔK_M may be calculated since $K_{CO_2}^{Ringer}$ is known for each flask and K_M may be experimentally determined. Assuming that

ΔK_M is proportional to the weight (w) of tissue, the retention factor per milligram of tissue ($\Delta' K_M$) may be calculated as follows:

$$(3) \qquad \Delta' K_M = \frac{\Delta K_M}{w}.$$

Knowing $\Delta' K_M$ for each vessel, the value of K_M for a particular weight of tissue may be calculated as follows:

$$(4) \qquad K_M = K_{CO_2}^{Ringer} + (w \times \Delta' K_M).$$

From the value of K_M for a particular weight of tissue and vessel, the volume of CO_2 in cubic millimeters produced during the experimental period will follow by rearrangement of equation (1):

$$(5) \qquad X_{CO_2} = hK_M.$$

The value of $\Delta' K_M$ for each vessel was determined in several experiments using quantities of tissue which varied from 56 to 116 mgm.

TABLE 1

Values of K_M for 100 mgm. of muscle calculated from experimentally determined values of $\Delta' K_M$

	VESSEL NUMBER						
	2	3	4	5	6	7	WEIGHT OF TISSUE USED
	$K_{CO_2}^{Ringer}$						
	1.624	1.632	1.573	1.647	1.560	1.543	
							mgm.
	2.014	1.890	1.839	1.857	1.744	1.796	62
	1.932	1.873	1.854	1.935	1.812	1.824	115
	1.887	1.903	1.799	1.903	1.810	1.836	116
K_M	1.961	1.901	1.868	1.935	1.838	1.792	115
	1.896	1.881	1.812	1.889	1.822	1.865	116
	1.929	1.944	1.820	1.912	1.820	1.757	100
	1.926	1.919	1.897	1.858	1.850		56
			1.777		1.764		56

RESULTS. In order that the results obtained from the determinations of the acid retention factor may be compared more easily, the K_M values for 100 mgm. of tissue have been calculated from equation (4) above, using the values of $\Delta' K_M$ which were experimentally determined with different weights of tissue. Those values have been collected in table 1. It will be seen that the K_M values for each flask have a maximum variation of 3.6 to 6.3 per cent. This proves the original assumption that the reten-

tion is proportional to the weight of tissue used if all other factors are kept constant and establishes the validity of the application of this formula in our calculations.

Table 2 summarizes the results obtained on the muscles of forty-seven frogs. The choline esterase activity is expressed, using the nomenclature of Nachmansohn (6), as the milligrams of acetylcholine hydrolysed by 100 mgm. of tissue in 60 minutes ($Q_{Ch.E}$). It will be seen that the second fifth has a rate of hydrolysis which is three times that of the pelvic fifth, and the third fifth has a rate which is a little more than twice that of the pelvic fifth.

The average of four experiments with the frog's sciatic nerve gives a $Q_{Ch.E}$ of 0.677, or nearly seven times that of the pelvic fifth of the sartorius muscle.

TABLE 2

Hydrolysis of acetylcholine by portions of frog's sartorius muscle $Q_{Ch.E}$ = *mgm. acetylcholine hydrolysed/100 mgm. muscle/60 min.*

NUMBER	PELVIC FIFTH		SECOND FIFTH		THIRD FIFTH	
	Muscle weight	$Q_{Ch.E}$	Muscle weight	$Q_{Ch.E}$	Muscle weight	$Q_{Ch.E}$
	mgm.		*mgm.*		*mgm.*	
1	128	0.076	108	0.221		
2	137	0.081	119	0.317		
3	140	0.100	123	0.328	122	0.220
4	142	0.060	132	0.265	132	0.205
5	165	0.133	141	0.285	123	0.192
6	162	0.157	140	0.391	119	0.267
Average.....		0.101		0.301		0.221

DISCUSSION. The results reported here on Rana pipiens agree qualitatively with those obtained by Marnay and Nachmansohn on Rana esculenta, but they do not agree quantitatively, for Marnay and Nachmansohn found that the $Q_{Ch.E}$ values for the pelvic, second and third fifths of the sartorius for Rana esculenta were 0.135, 0.795 and 0.404 respectively, while the corresponding values for Rana pipiens were 0.101, 0.301 and 0.221, or a difference of 26 per cent, 62 per cent and 45 per cent respectively. There is also a relative difference in the esterolytic activity of the muscles from the two frogs, since the $Q_{Ch.E}$ value for the second fifth of Rana esculenta is approximately seven times that of the pelvic fifth, while the $Q_{Ch.E}$ value for the second fifth of Rana pipiens is three times that of the pelvic fifth; and the value for the third fifth of Rana esculenta is four times that of the pelvic fifth, while for Rana pipiens the value of the third

fifth is a little more than twice that of the pelvic fifth.   These differences in the corresponding values for the muscles of Rana esculenta and Rana pipiens suggest that the distribution of motor nerve endings in the two muscles is not the same.

In a later paper (6) Nachmansohn says: "Marnay and Nachmansohn have shown that there exists an extremely high concentration of choline esterase at the nerve endings of voluntary muscle and that during the refractory period of voluntary muscle, in a few milliseconds, the amount of acetylcholine required by the theory of chemical transmission can well be hydrolysed at the nerve endings."   The amount of acetylcholine which must be liberated at the nerve ending to satisfy the theory of chemical transmission is not known to us, and, furthermore, the amount of acetylcholine actually liberated at the nerve ending in the sartorius muscle of the frog has not been determined; until these quantities are known it is impossible to say that the choline esterase which is also present can effect its hydrolysis in a few milliseconds.

Clark et al. (7) have shown that the activity of choline esterase depends upon its concentration in solution.   Marnay and Nachmansohn assumed a nerve ending volume of $\frac{1}{1000}$ of the muscle volume, and later, in answer to the objections of Clark et al., Nachmansohn (6) assumed a nerve ending volume of 1000 μ^3, in which the enzyme would have a concentration high enough to permit maximum activity.   Until proof is presented to the contrary, it appears that it must be assumed that the esterase and acetylcholine are acting in the volume occupied by the motor end-plate of the nerve fiber.   From measurements which we have made of the drawings (with magnification given) by Kühne (8) of the end-plates in skeletal muscles of Rana esculenta, it is estimated that this volume is several times more than 1000 μ^3.   Until such measurements of volume are accurately made, it is not possible to say that the choline esterase which is present in the end-plate has a concentration sufficiently high to give the enzyme maximum activity.

Although it is evident that choline esterase is concentrated to some extent at the motor nerve ending, one is unable to say yet how effective the enzyme is in hydrolysing the acetylcholine which is liberated there during stimulation.

SUMMARY

1. The method of Warburg, Kubowitz and Christian for calculating the acid retention of serum has been applied to tissue suspensions.

2. The rate of hydrolysis of acetylcholine by the second fifth of the sartorius muscle of Rana pipiens is three times that of the pelvic fifth.   The rate of hydrolysis of the third fifth is approximately twice that of the

pelvic fifth.   There is an absolute and relative difference, in this respect, between the muscle of Rana pipiens and Rana esculenta, which suggests a difference in the distribution of motor endings in the two cases.

3. It is not yet possible to state that the choline esterase which is present at the motor nerve ending will hydrolyse in the refractory period the acetylcholine which is liberated there.

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CHEMICAL COMPOSITION OF UTERINE SECRETIONS[1]

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Many studies have been made on the nature of uterine epithelium and the effect of endocrine secretions on it, but no chemical analyses have been reported on the product of these secretory cells, the uterine fluid. The amount of this fluid varies in different species. Long and H. M. Evans (1922) first described distention of the normal rat uterus with fluid which accumulates in pro-oestrus and oestrus, with discharge during late oestrus. Warren (1938) found that about 18 times as much uterine fluid was present in pro-oestrus as in dioestrus; 3 weeks following surgical occlusion of the cornua in rats the average total amount of fluid in the uterus was 5.5 cc. De Marco (1930) using uterine fistulae in dogs found that pilocarpine stimulated the flow of uterine fluid so that 0.5 to 1.75 cc. were obtained per hour. E. I. Evans (1933) found scant amounts of fluid in the canine uterus. Schockaert and Delrue (1938) determined the pH of the cervical mucus in women.

METHODS. Chemical studies were made on the uterine secretion of 5 dogs, 6 rabbits and 18 rats.

Physiological methods. Ether anesthesia was used for all surgical procedures, which consisted in preparation of uterine fistulae or closed segments; except in the dogs with fistulae, all of the uterine fluids were obtained by opening the abdomen surgically and aspirating the uterus with a fine needle.

In 3 dogs uterine fistulae were made according to the method used by Reynolds (1930) for rabbits, and following healing, freshly prepared pilocarpine hydrochloride, 6 mgm. in saline, was injected intravenously as a secretory stimulant. No fluid was obtained from the cervices unless the dog was in heat, so that stilboestrol 0.1 to 1.0 mgm. was injected intramuscularly in all dogs daily. Subsequently in 2 of these animals a closed uterine segment was prepared by ligation of the uterus proximally and distally. In 2 additional dogs similar closed uterine segments were prepared by proximal and distal ligation of the uterine horns. Fluid was obtained from the closed segments by aspiration at weekly intervals.

Similar methods were used in the rabbits and rats, but insufficient fluid was obtained from uterine fistulae for chemical analyses so that secretion was secured from the closed segments in most cases. In these groups uterine secretion was obtained: 1, following uterine ligation alone (2 rabbits, 5 rats); 2, uterine ligation and estrogen injection (4 rabbits, 10 rats); and 3, estrogen injection without ligation (1 rabbit, 5 rats). In 5 rats fluid was removed from the normal uterus during oestrus without preliminary ligation and in 5 rats estrogen was injected beginning 3 weeks following bilateral ovariectomy. As estrogenic material either stilboestrol 0.1 mgm. or estrone 10 I.U. in oil was injected daily.

Chemical methods. Water determinations were made by drying known weights of the fluid to constant weight at 105°C. Chloride was determined by the method of Van Slyke (1923) as modified by Wilson and Ball (1928); sodium, by the method of Butler and Tuthill (1931); calcium, by the method of Kramer and Tisdall (1921) as modified by Clark and Collip (1925); and potassium, by the method of Shohl and Bennett (1928) as modified by Eichelberger (1938). Glucose was determined by the method of Shaffer and Hartman (1921) as modified by Somogyi (1926); inorganic phosphorus, by the method of Fiske and Subbarow (1925). Total nitrogen and non-protein nitrogen were determined by the micro-Kjeldahl method of Koch and McMeekin (1924) and the proteins by multiplying by 6.25 the total nitrogen corrected for non-protein nitrogen. pH was determined with the glass electrode and the total CO_2 by the method of Van Slyke and Neill (1924) on fluid collected under oil.

RESULTS AND DISCUSSION. Uterine fluid was found to be limpid, slightly opalescent and contained occasional epithelial cells on microscopic examination. As compared with prostatic secretion obtained by pilocarpine stimulation after isolation of the prostate gland according to the technique of Huggins, Masina, Eichelberger and Wharton (1939), the fluid was scant in amount. The rate of secretion in dogs with uterine fistulae after pilocarpine stimulation was 0.2 to 0.4 cc. per hour; thus only several chemical determinations were made on the fluid obtained at any one sitting of a few hours' duration.

In animals with closed uterine segments, larger quantities of fluid were obtained. The volume obtained from the 2 horns of the uterus of the rat varied from 0.2 to 1.4 cc.; of the rabbit, from 1 to 5.5 cc.; and from 7 to 17 cc. in the dog.

The chemical composition of uterine fluids is shown in table 1. Points of similarity in the fluids from the dog, rabbit and rat uteri were the high content of water and sodium and the low protein content. Phosphate and glucose were present in very small amounts or absent.

The uterine fluids of rat and rabbit resembled each other and were characterized by an alkaline pH, a high total CO_2 content, with the chloride

concentration being normal with respect to plasma. The uterine fluid of the dog differed from these in having an acid pH, a low total CO_2 content and a high chloride concentration.

TABLE 1

Chemical composition of uterine fluid

	H₂O	pH	CO₂	TOTAL N	NPN	PRO-TEIN	Cl	Na	Ca	K	GLU-COSE	INOR-GANIC P
Dog												
	gm.		mM	gm.	gm.	gm.	mM	mM	mM	mM	mgm.	mM
1†	986			0.30	0.19	0.70	180	170	4.7	4.1	0	0
	985			0.40	0.13	1.72	190	140	2.8	6.4	80	0
2†	986	6.21	3.0	0.50	0.30	1.25	149	165			0	0
3‡	975	6.26	3.8		0.11		149	164				
4‡	986	5.91	2.6	1.09	0.27	5.12	156	169			70	0.03
5‡	986	6.05	2.6	1.76	0.17	9.94	157	161			70	0.03
Average*........	984	6.09	3.0	0.8	0.20	3.8	167	162	3.5	5.2	0–80	0–0.03
Rabbit												
Uterine ligation	983	7.73	55.3	0.67	0.44	1.41	91	166	7.9	6.1	0	0
	981	7.80	48.2	0.94	0.29	4.0	99	150	1.5		160	0.14
Estrone	980	7.90					98	191				
	976	7.73	51.2									
Uterine ligation & estrone	976	7.75	48.4				103				100	0.20
Average*........	979	7.78	53.6	0.8	0.37	2.7	98	158	4.7	6.1	0–160	0–0.20
Rat												
Stilboestrol injections	985	7.68					106	215				
Uterine ligation & stilboestrol	979	7.66	61.8	1.32	0.29	6.53	101	123	1.5	4.3	150	0
				0.47		1.12	98					
Ovariectomy and estrone	990	7.32					92					0
Average*........	982	7.55	61.8	1.0	0.29	5.1	98	169	1.5	4.3	150	0

Values expressed per liter of fluid.

* Averages represent all of our data, not merely those represented in this table.

† Uterine fistula, pilocarpine stimulation.

‡ Uterine ligation, pilocarpine not used.

The distribution of electrolytes in uterine fluids is thus different from that in serum and transudates, demonstrating that it is formed by the process of secretion. The low concentration of protein requires readjustments to be made to secure osmotic equilibrium. In all the animals sodium

was the cation present in largest amounts; the principal anion in fluid from the dog was chloride, bicarbonate being low; while in rat and rabbit the chloride was at serum level and the bicarbonate concentration was high.

It is noteworthy that the various procedures employed resulted in no differences in chemical composition in the same species and especially that the fluid obtained after stimulation with pilocarpine in dogs with uterine fistulae resembled that in the closed segment without drug injection.

The similarity of rat and rabbit uterine fluids has been discussed above. The uterine fluid of the dog, differing from these, resembles closely, however, certain fluids of the genital tract in the male, notably prostatic fluid obtained after pilocarpine stimulation in dogs and spermatocele fluid obtained from the epididymis in man (table 2). Thus, there are recurrent

TABLE 2

Comparison of chemical composition of genital tract fluids

	H_2O	pH	CO_2	TOTAL N	NPN	PRO-TEIN	Cl	Na	Ca	K	GLU-COSE	INOR-GANIC P
	gm.		*mM*	*gm.*	*gm.*	*gm.*	*mM*	*mM*	*mM*	*mM*	*mgm.*	*mM*
Rabbit uterine fluid............	979	7.78	53.6	0.8	0.37	2.7	98	158	4.7	6.1	0-160	0-0.20
Rat uterine fluid.	982	7.55	61.8	1.0	0.29	5.1	98	169	1.5	4.3	0-150	0
Dog uterine fluid.	984	6.09	3.0	0.8	0.20	3.8	167	162	3.5	5.2	0-80	0-0.03
Dog prostatic fluid*..........	981	6.16	0.8		0.22	8.3	160	159	0.3	5.1	0-300	
Human sperma-tocele fluid†....		7.13	6.3		0.26	5.5	139		2.3		0-tr.	0

Values expressed per liter of fluid.

* Following pilocarpine stimulation: data of Huggins, Masina, Eichelberger and Wharton (1939).

† Data of Huggins and Johnson (1933).

regional chemical patterns in the fluids of the genital tract, one pattern seen in male and female dogs and the male human individual, while another is found in female rats and rabbits. The low content of glucose and phosphate in uterine fluids in the dog, rat and rabbit, in the prostatic fluid of dog and man, and in spermatocele fluid in man must be sharply contrasted with the high phosphate and glucose content of the human seminal vesicle first described by Huggins and Johnson (1933).

In the rat the uterine horns were found to be atrophic and collapsed 3 weeks after bilateral ovariectomy and became distended after estrogenic stimulation for 1 week. Analyses of the fluid showed it to be essentially the same as that in rats which had not been spayed or injected with estrone, demonstrating that estrogen alone as a hormone can produce in the uterus this characteristic fluid.

CONCLUSIONS

Uterine fluids obtained from rats and rabbits resemble each other chemically. They have points of similarity to and difference from uterine fluid of the dog. The points of similarity are the high content of water and low concentrations of protein, glucose and inorganic phosphate; the principal cation is sodium. The fluids differ in that in the dog the pH is acid, total CO_2 content is low and chloride relatively high, while the uterine fluid of rats and rabbits is characterized by an alkaline pH, high total CO_2 content, with the chloride concentration at the plasma level.

No significant differences in chemical composition were detected in fluid obtained by pilocarpine stimulation from uterine fistulae or in closed segments without stimulation. Injections of estrogen alone in spayed rats produced the uterine fluid typical of the normal rat.

Certain chemical patterns are seen in the similarity of fluids from various regions of the genital tracts of male and female animals. Thus rat and rabbit uterine fluids are identical from an electrolyte standpoint and differ from dog uterine fluid, dog prostatic fluid obtained after pilocarpine stimulation and human spermatocele fluid, the last three resembling each other.

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EFFECTS OF MAGNESIUM ON THE NERVOUS SYSTEM IN RELATION TO ITS CONCENTRATION IN SERUM[1,2]

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A series of cardiovascular changes during the slow intravenous injection of magnesium salts has been previously described (11, 13), each event appearing during a characteristic range of concentration of magnesium in the serum. The relation between concentration on the one hand and changes in reflex activity and in neuromuscular transmission on the other forms the subject of the present study.

METHODS. Decerebrate cats were usually employed, with supplementary experiments on neuromuscular transmission in animals anesthetized with sodium amytal. The muscles to be studied were isolated by appropriate nerve and tendon section and the contraction recorded with a torsion wire myograph. The leg was held rigidly by drills fixed in the bone. A thyratron stimulator was used to give single or repetitive supramaximal break shocks at various frequencies. Magnesium sulfate in isotonic solution was injected slowly into the femoral vein at a constant rate. Samples of serum obtained at intervals were analyzed for magnesium by a method previously described (14). Concentrations corresponding to particular effects were obtained by interpolation between determined values. In some experiments the ureters were tied to prevent urinary excretion. Artificial respiration was employed in all experiments after natural respiration failed and throughout the experiment when the thorax was opened.

RESULTS. The results of the entire series of experiments are summarized in table 1. The figures in parentheses represent individual values in different experiments. In figure 1 the concentrations at which repetitive shocks to the motor nerve just failed to produce a response are plotted against frequency of stimulation.

Single twitches disappeared at concentrations ranging from 6 to 14 mEq

per liter. At this level the muscle still responded to even the slowest tetanic stimuli. Twitches obtained from the quadriceps, the gastrocnemius-soleus and from isolated strips of diaphragm all gave similar values. In experiments in which two of these muscles were stimulated simultaneously the twitches disappeared at the same time in both muscles. Concentrations of magnesium sufficient to induce failure of response to tetanic stimuli (fig. 1) varied with the frequency of stimulation, fast stimuli still being effective at concentrations far above those at which slower stimuli had ceased to act. Tetani of a given frequency failed in all different muscles at the same time, just as did the twitches.

Two special characteristics of partial neuromuscular block were noted. 1. With partial neuromuscular block due to magnesium the characteristic

TABLE 1

The concentration of serum magnesium required for extinction of neuromuscular transmission and certain reflexes

CONCENTRATION OF MAGNESIUM IN SERUM	FAILURE OF RESPONSE TO STIMULATION OF MOTOR NERVE. RATE OF STIMULATION	FAILURE OF REFLEX
*mEq per l.**		
6–14	Single twitches	Tendon jerks (S, 10, 12, 13)
12–15	Tetani at 20 per sec.	Crossed extensor and shortening reflexes (S, 14, 22)
15–20	Tetani at 40 per sec.	Natural respiration (17, 19, 20, 21, 22)
20–25	Tetani at 60 per sec.	
25–30	Tetani at 100 per sec.	Vibrissa and prima reflexes (20, 26, 27, 29)
30–35	Tetani at 200 per sec.	Corneal reflex (29, 30, 32)

* In each experiment the sequence of neuromuscular and reflex failure given in this table took place as progressively higher levels of serum magnesium were attained. The overlapping of serum magnesium values is due to variations in the absolute level in the several experiments.

self inhibition ("Wedensky" inhibition) of the tetanus appeared at lower frequencies and was more pronounced (figs. 2A, 2B). 2. Twitches which had previously been extinguished reappeared for a time following a tetanus. This "enhancement" or "post-tetanic potentiation" is illustrated in figure 2B.

In every case reflexes were studied simultaneously with the neuromuscular twitches and tetani. Therefore the point of extinction of each reflex was related not only to the serum magnesium value but also to the cessation of neuromuscular activity in each individual experiment. The concentrations were scattered over a considerable range. The knee jerk and other tendon jerks were the first reflexes to disappear, their failure coinciding with that of the neuromuscular twitches. A tetanic contraction

just after the knee jerks had disappeared caused them to reappear for a time, just as it caused the reappearance of direct neuromuscular twitches. No changes in other reflexes could be detected at this level. The crossed-extensor and shortening reflexes failed at somewhat higher concentrations,
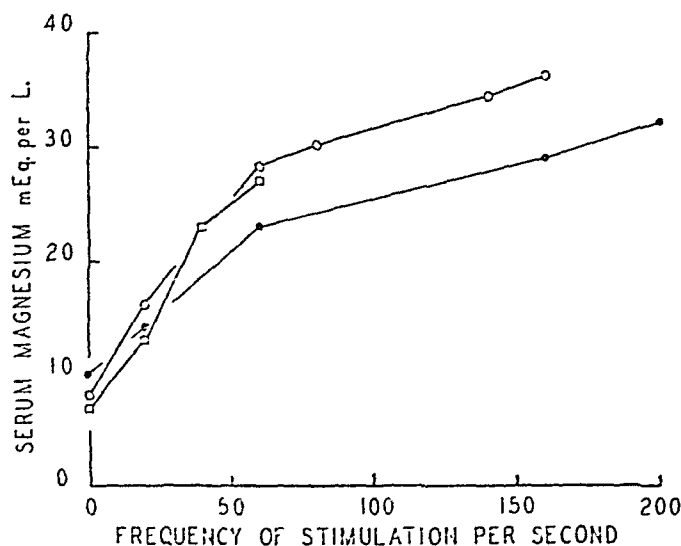

Fig. 1   Fig. 2

Fig. 1. The concentrations of magnesium at which tetanic contraction disappeared are plotted against the frequency of stimulation in 3 individual experiments. The more rapid the stimulation, the higher is the concentration of magnesium necessary to block a response. The values at 0 are those for single twitches.

Fig. 2A. Figure 2A represents a control experiment in which no magnesium had been injected. Each number indicates the frequency of stimulation of the tetanic contraction recorded just below it. Self inhibition, or "Wedensky" inhibition, occurs first at 60 per second. The interval between the initial contraction and the onset of inhibition becomes progressively shorter as the frequency of stimulation increases from 60 to 260 per second.

Fig. 2B is taken from the same experiment after magnesium had been injected until the concentration in serum attained 14 mEq per liter. Each numeral indicates the frequency of stimulation of the tetanic contraction recorded below it. Ordinates represent the tension developed by the muscle. Single shocks and slower frequencies of stimulation elicit very little response, and at higher frequencies Wedensky inhibition is much more marked than in the control. At the extreme right is the record of the tetanic response to a stimulus at 20 per second at the close of the experiment. This tetanus was followed for a time by a return of the response to single stimuli, i.e., by "post-tetanic enhancement."
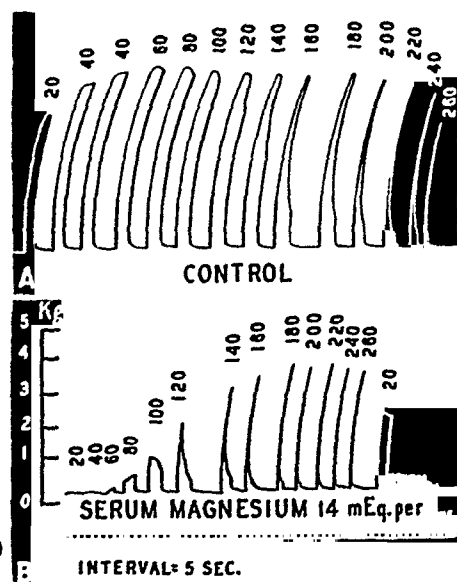
corresponding to the level of failure of slow neuromuscular tetani (frequency of about 20 per second). At a little higher concentration natural respiration failed; simultaneously neuromuscular tetani at the rate of 30 to 50 per second failed. The corneal reflex, the pinna reflex and the

vibrissa reflex (protrusion of vibrissae following stimulation of the ear) all persisted long after respiratory failure, disappearing only when tetani at 60 to 100 per second failed.

It was found that reflexes which had disappeared could be brought back for a time if a brief neuromuscular tetanus were applied to the motor element of the arc. This is of course the same "enhancement" as that previously noted as characteristic of partial neuromuscular block. The height of contraction of muscles directly stimulated was invariably found to be unaffected by magnesium injection.

DISCUSSION. The sequence in which the reflexes disappear (table 1) is quite different from that following the administration of a volatile anesthetic or a barbiturate. With these anesthetics the knee jerk is especially resistant, while the corneal reflex disappears relatively early. Respiration, which disappears so early after magnesium, disappears late in the course of the action of the ordinary anesthetics (8). This difference in effect on reflexes makes it probable that the locus of action of magnesium differs from that of most anesthetics.

The order of disappearance of reflexes after magnesium is, however, correlated with the type and rate of discharge characteristic of the motor neurones comprising the motor element of the reflex arc. The reflexes with slow frequencies of discharge disappear first, those with higher frequencies later. Thus the knee jerk and other tendon reflexes are largely single twitches, each twitch being produced by a single discharge in most of the motor neurones taking part in the reflex (7, 10). The flexion reflex, another twitch-like reflex (5, 7) is known to be abolished at levels of serum magnesium comparable with those reported here for twitches and tendon jerks (3). The crossed-extensor reflex and the shortening reaction in quadriceps or soleus is largely mediated by rhythmic discharges at rates varying from 10 to 20 per second (2, 5, 6, 10). Respiration is mediated in the diaphragm by impulses from phrenic motor neurones discharging 25 to 30 times per second (1), with normal breathing, somewhat more rapidly with forced breathing. The rate of impulses in muscles of the eye, ear and vibrissae is not known, but there is indirect evidence that they may be 100 or more per second (4).

If these characteristic rates of discharge of the different reflexes are compared with the concentrations of magnesium at which neuromuscular tetani of the same rate fail (table 1), an excellent correlation is disclosed. Thus twitch-like reflexes fail when twitches fail, slow tetanic reflexes fail with slow neuromuscular tetani, and rapid tetanic reflexes and rapid neuromuscular tetani disappear together when much higher levels are attained. The suggestion is clear that reflexes fail because of neuromuscular block rather than because of an action of magnesium on the central nervous system. This interpretation is strengthened by the observation that

twitch-like reflexes may be made to reappear by neuromuscular tetani of their motor elements. If failure of the reflexes were due primarily to block at the central synapse, no procedure affecting only the peripheral neuromuscular mechanism could possibly restore the reflex.

Experiments such as these do not in any way exclude an effect of magnesium on the central nervous system. The demonstration by Peck and Meltzer (12) that anesthesia may be produced in man by the injection of magnesium is unequivocal evidence that such a central effect does exist. They do, however, indicate that magnesium affects muscular activity mainly through its action on the neuromuscular junction and not through its action on the central nervous system.

The possibility must be considered that the curare-like action of magnesium excess is in some way the converse of the tetany of magnesium deficiency. The tetany of animals depleted of magnesium is peculiar because it is not necessarily associated with low magnesium in serum, is not immediately relieved by injection of magnesium and is not prevented by curare (9). Failure to abolish these convulsive seizures by curare places the site of disturbance in the muscles themselves, beyond the myoneural junction. Since the response of muscles stimulated directly rather than through their motor nerves is unaffected by excess of magnesium, the locus of action of magnesium excess must be the myoneural junction itself. This difference in point of action suggests that the phenomena of magnesium excess and those of magnesium deprivation are not intimately related.

The conclusions drawn in this study are not in agreement with those of Bryant, Lehmann and Knoefel (3). These authors, using the spinal dog, found that the flexor response to single induction shocks failed while the skeletal muscle still responded to stimulation of its motor nerve. From this observation they concluded that the action of magnesium is primarily central. Our experiments show that this conclusion would be justified only if the type and rate of stimulation of the skeletal muscle is identical with that produced by the natural reflex, since the level of extinction of response to neuromuscular stimulation varies so much with frequency of stimulation. These workers do not specify the type of stimulation used to stimulate the skeletal muscle, so it is not possible to decide whether or not this is actually the cause of the apparent discrepancy between their results and ours.

CONCLUSIONS

1. Concentrations of magnesium at which neuromuscular transmission failed and concentrations at which various reflexes disappeared were determined in the cat.

2. The greater the frequency of stimulation, the higher is the concentration of magnesium necessary to block transmission.

3. It is probable that magnesium affects muscular activity by blocking neuromuscular transmission rather than by means of its depressant action on the central nervous system.

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COMPARATIVE PARENTERAL AND ORAL ASSAYS OF ADRENAL CORTICAL HORMONE SUBSTANCES

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An early report of the oral treatment of adrenal insufficiency with adrenal cortex was given by Osler in 1896 (1). He apparently successfully treated a case of Addison's disease with a glycerol extract of pig adrenals. With the preparation of epinephrine-free biologically active extracts of the adrenal cortex, adrenal insufficiency has for the past several years been quite commonly treated by parenteral administration. Grollman (2) reported the successful treatment of adrenal insufficiency in rats, also, by the oral administration of a charcoal adsorbate.

In a previous report (3) we described a rat method of assaying adrenal cortex extracts and noted that adrenalectomized rats could be successfully maintained by administration of the extracts in the drinking water, and that such administration gave better growth response than subcutaneous injections. Later reports (4, 5) present further evidence that the adrenalectomized rat is a suitable test animal for the assay of adrenal cortical extracts, and that these extracts, orally, are capable of maintaining the test animal, as Thorn et al. (6) have also found for the adrenalectomized dog. In one of these reports (5) an "oral rat unit" is described.

Recent clinical reports (7, 8) have called attention to the effectiveness in the human subject of orally administered adrenal cortical hormone preparations. Some clinical data (7) indicate that, on the basis of gland equivalence, the administration of the oral preparation is more effective than parenteral injection of the aqueous sterile solution. A study of parenteral and oral assays of adrenal cortical hormone material is therefore desirable.

The present study is concerned with a direct comparison of parenteral and oral assays of adrenal cortex concentrates in the 4-weeks-old adrenalectomized white rat. The comparative effectiveness of the more active crystalline substances of the adrenal cortex, corticosterone, dehydrocorticosterone, and desoxycorticosterone, has also been studied, and the cortical-hormone activity in rat units quantitatively determined by subcutaneous injection, as well as by administration by stomach tube.

EXPERIMENTAL. The composite extracts of the adrenal cortex which were used and which so far as we know contain all the cortical hormone

active substances present in the gland, were prepared from beef suprarenal glands by the process previously reported (9).

For the determination of the parenteral and oral activity of desoxycorticosterone acetate, the synthetic material was used. The corticosterone and dehydrocorticosterone were obtained from the least water-soluble fraction of a gland extract by procedures previously described (10). They were isolated in pure form and identified by their melting points, analyses, and specific rotations.

METHODS. The composite extracts were assayed parenterally as previously described (3). For the determination of their oral effectiveness they were assayed both by administration in the drinking water and by administration by stomach tube. For assay by drinking water administration, the daily dose of hormone was added to the estimated amount of water the rat would drink over a 24 hour period. All assays were carried out over a 20 day period, hormone administration being withdrawn on the 20th day. The rat units per cubic centimeter of extract were estimated as described in the previous report, one rat unit being the minimum daily dose of hormone which, administered daily for 20 days to 4-weeks-old male rats weighing 50 to 60 grams, is sufficient to protect at least 80 per cent of the rats and produce an average growth of at least 20 grams for the 20 day period.

Since administration in the drinking water depends on the voluntary consumption by the assay animal, it might be objected that quantitative results can not be obtained, and that a "minimum daily dose" can not therefore be accurately determined. We have, therefore, also carried out assays by administering the extracts once daily by stomach tube. Since in determining the rat unit parenterally one daily subcutaneous injection is given, the most comparable oral determination of rat units can be obtained by one daily administration by stomach tube. We find that a small gauge soft rubber catheter can easily be pushed down a small rat's esophagus, and that oral administration can thus be as easily carried out as subcutaneous injections.

The care of the rats, the diet, and the technique of removal of the adrenal glands are the same as previously described (3).

RESULTS. In table 1 is given a summary of the assays of several adrenal cortex extracts. It can readily be seen that, whether the extracts are assayed parenterally, by subcutaneous injection, orally, by either stomach tube administration or drinking water administration, the result in rat units per cubic centimeter of extract is the same. Adrenal insufficiency in the young adrenalectomized rat can therefore be controlled equally as well by oral administration of cortical extracts as by parenteral administration. For each assay in table 2 at least 10 rats were used. The routine procedure was to administer 0.3 and 0.4 cc. of the extracts per

rat per day for the 20 day period. In some cases 0.2 cc. doses were also given. If an extract failed to pass the test at 0.3 cc., but passed at 0.4 cc., it is reported as having 3.0 rat units per cc., the average of 3.3 and 2.5 being practically 3. If the extract passed well at both doses, 0.3 cc. and 0.4 cc., it is reported to have 4.0, the average of 5 and 3.3, rat units per cc. Nearly all extracts failed to pass the test at 0.2 cc. per rat per day, except in a few instances (51–5 and 54–5). Any extract which barely passed the test at 0.3 cc. is reported as containing 3.3 rat units per cc.

In table 2 are given the results of the parenteral assays of the pure crystalline substances, corticosterone, dehydrocorticosterone, and desoxycorticosterone. The substances were administered in olive oil solution,

TABLE 1

Comparison of parenteral and oral assays of adrenal cortex extracts

PREPARATION	PARENTERAL ASSAYS	ORAL ASSAYS, DRINKING WATER ADMINISTRATION	STOMACH TUBE ADMINISTRATION
	rat units per cc.	*rat units per cc.*	*rat units per cc.*
51–5	5.0	5.0	
54–5	4.0	5.0	
47–5	4.0	<5.0	
288–5	3.3	4.0	4.0
43A–6	3.0	3.0	
44–6	4.0	4.0	
45A–6	3.0	3.0	3.3
48–6	3.0	3.0	
52–6	4.0	4.0	
55–6	3.0	3.0	
291–6	4.0	4.0	4.0
9–6	3.0	3.0	3.3
13–6	4.0	4.0	4.0
29–6	3.0		4.0
292–7	3.0		3.0
66A–6	4.0	4.0	

and the concentrations were such that at all dosage levels 0.2 cc. of the oil solution was injected. It is apparent from the tabulated assay results that, parenterally, corticosterone assays 6 rat units per mgm., while according to our available data dehydrocorticosterone assays about 4 rat units per mgm. Desoxycorticosterone acetate, however, assays 35 rat units per mgm. by subcutaneous injection.

In table 3 are tabulated the results of the oral assays of the pure substances. For these oral assays the substances were also dissolved in olive oil and again the concentrations were adjusted so that the one volume of 0.2 cc. was administered at all dosage levels. As with the composite crude extracts, so with corticosterone, the parenteral and oral assays in

rat units are the same. Dehydrocorticosterone apparently is somewhat more active orally than by injection, assaying orally 6 rat units per mgm. and parenterally 4 rat units per mgm. Desoxycorticosterone acetate, however, differs very strikingly with respect to oral effectiveness. Whereas parenterally the substance assays 35 rat units per mgm., orally it fails to

TABLE 2

Parenteral assays of crystalline adrenal cortex substances

SUBSTANCE	DAILY DOSE	NUMBER OF RATS INJECTED	NUMBER OF RATS SURVIVING 20 DAYS	AVERAGE GROWTH IN 20 DAYS	AVERAGE SURVIVAL AFTER LAST INJECTION	RAT UNITS PER MGM. OF SUBSTANCE
	mgm.			grams	days	
Corticosterone	0.25	5	5	29	4.0	>4⎫ 6
	0.125	5	2	13.5	2.0	<8⎭
Dehydrocortico-sterone	0.5	3	2	29	6.5	>2⎫
	0.25	5	4	21	4.0	4⎬ 4
	0.125	3	0			<8⎭
Desoxycortico-sterone acetate	0.05	5	5	34	5.0	>20⎫
	0.033	5	5	34	6.2	>30⎬ 35
	0.025	5	3	30	8.0	<40⎭

TABLE 3

Oral assays of crystalline adrenal cortex substances

SUBSTANCE	DAILY DOSE	NUMBER OF RATS INJECTED	NUMBER OF RATS SURVIVING 20 DAYS	AVERAGE GROWTH IN 20 DAYS OF 20 DAY SURVIVALS	AVERAGE SURVIVAL AFTER LAST INJECTION	RAT UNITS PER MGM. OF SUBSTANCE
	mgm.			grams	days	
Corticosterone	0.25	9	9	26	7.0	>4⎫ 6
	0.125	5	0			<8⎭
Dehydrocortico-sterone	0.25	5	5	25	6.2	>4⎫ 6
	0.125	5	0			<8⎭
Desoxycortico-sterone acetate	0.033	5	0			<30
	0.10	5	0			<10
	0.20	5	0			<5
	1.0	5	3	11	6	<1

pass the test even at 1 rat unit per mgm. This substance is therefore less than one-thirty-fifth as effective orally as parenterally in the young adrenalectomized rat. This animal therefore can completely utilize orally administered corticosterone, while desoxycorticosterone acetate administered orally can scarcely be used at all. This is very interesting in view of the fact that the substances are so nearly alike chemically.

To determine whether desoxycorticosterone is excreted in the feces and is therefore unabsorbed, the fecal material of the 5 rats that received 1 mgm. daily of the synthetic by stomach tube was collected for one week, from the 7th to the 14th day, and extracted with acetone. The acetone soluble material was dissolved in ether, and the ether solution washed first with dilute HCl, then with 1 per cent Na_2CO_3 solution, and finally with water, after which it was dried with anhydrous $MgSO_4$. The ether soluble substance was transferred to 22 cc. olive oil and the solution injected subcutaneously at 0.2 cc. per rat per day into a group of 5 adrenalectomized rats. The average survival period of this group of 5 rats was 7.8 days as compared with 7 days for a control group. There was therefore no adrenal cortical hormone activity in this fecal extract, and since the desoxycorticosterone acetate assayed 35 rat units per mgm. when administered subcutaneously, there certainly must have been excreted less than 3.3 mgm. of the substance by the entire group of 5 rats for the 7 day period. Since 35 mgm. had been administered over this same period, it would appear that most of the substance was destroyed in the gut.

DISCUSSION OF RESULTS. Difficulties of oral administration by mixing extracts with the food have been pointed out (5). Since mixing the daily dosage with the drinking water or food makes the hormone intake too dependent "upon the voluntary consumption of food by the assay animal," we have administered the extract by stomach tube. By such single daily oral administration the minimum requirement of the composite gland extract is the same as by single daily subcutaneous injection. The rat unit as previously defined by us[1] can therefore also be used to express adrenal cortical hormone activity by oral assay. It only becomes necessary when stating rat units of a certain extract or substance to designate whether the substance was assayed orally or parenterally, since we know of at least one single pure substance which is less active orally than parenterally.

According to Grollman (11), 1 mgm. of desoxycorticosterone acetate per rat per day, orally or parenterally, is effective in maintaining normal growth in adrenalectomized rats. Our data do not support such a conclusion. According to our rat method of assay, this synthetic substance is less than one-thirty-fifth as active orally as parenterally. It was found orally to assay even less than 1 rat unit per mgm. DeFremery and Spanhoff (12) have also reported that orally, "negative reactions only were obtained with even as much as 500 gamma daily." In this respect, therefore, the synthetic substance differs markedly not only from the natural

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[1] A rat unit is defined as the minimum daily dose of hormone which, administered daily for 20 days to 4-weeks-old rats weighing 50 to 60 grams, is sufficient to protect at least 80 per cent of the rats and produce an average growth of at least 20 grams per rat for the 20 day period.

extract, which is just as active orally as by injection, but also from the pure substances, corticosterone and dehydrocorticosterone, which are at least as active orally as parenterally.

SUMMARY

A method of assaying adrenal cortex extracts by stomach tube administration is described and it was found that by oral administration, the gland extracts assayed the same as by parenteral administration.

Corticosterone and dehydrocorticosterone are also as effective orally as parenterally.

Desoxycorticosterone assayed 35 rat units per mgm. parenterally by subcutaneous injection, but orally this substance assayed less than 1 rat unit per mgm.

Cortical hormone activity could not be demonstrated in the feces of the rats that had received desoxycorticosterone acetate by mouth. It appears, therefore, that this substance is destroyed in the gut.

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A NOTE ON THE QUESTION OF REFLEX ACTIVATION OF DORSAL ROOT DILATORS

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Since Bayliss (1902) published his observations on the activation of the dorsal root dilators to the legs by stimulation of the depressor nerve, the explanation and indeed the very existence of the phenomenon have been extensively debated by anatomists and physiologists. A review of the literature has recently been presented by Hinsey (1939). Reflex increases in the volume of a sympathectomized but otherwise intact leg of the dog have been reported by Fofanow and Tschalussow (1913) and by Tournade and Malméjac (1933). Rosenblueth and Cannon (1934), though they emphasize that reflex changes in the blood pressure of sympathectomized animals may be explained on the assumption that the dorsal roots are capable of reflex activation, were unable to demonstrate changes in limb volume which would account for the alterations in blood pressure. Downman et al. (1939) report that the vasomotor responses of cats' paws, recorded optically from plethysmographs, are abolished by sympathectomy. The evidence derived from manometric determinations of blood flow is divided between that of Bacq, Brouha and Heymans (1933) and of Derom and Grimson (1939), who obtained negative results, and that of Bishop, Heinbecker and O'Leary (1933), who, with a rather more intricate technique, obtained changes attributed by them to activation of dorsal root dilators.

Skin temperature experiments have been negative (Hinsey et al., 1934, 1938; Wybauw, 1938), a fact all the more important in view of the unanimity of opinion that the dorsal root dilators are almost exclusively distributed to the skin.

In view of the uncertainties, it seemed worthwhile to reinvestigate the experiments of Bayliss, especially since he himself emphasized the difficulty of demonstrating the phenomenon, and since he was unable to obtain completely satisfactory control observations. The following experiments were therefore designed to obtain records during vasomotor reflexes of the

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volume changes of a sympathectomized paw simultaneously with those of a completely denervated one.

METHOD. Cats or dogs anesthetized with dial (8 cats, 3 dogs) or urethane (8 cats, 3 dogs) were used. The lumbar sympathetic chains were removed from the third or fourth post-thoracic ganglion to the brim of the pelvis either on the day of the experiment or one to three weeks previously. Four of the cats were totally sympathectomized one to three months before the experiments. In order to minimize the fall of blood pressure produced by depressor stimulation, evisceration was performed in five of the cats and four of the dogs as recommended by Bayliss (1902). The adrenal glands were tied off in about one-third of the experiments. Denervation of a paw was effected by cutting the sciatic nerve at its emergence from the pelvis and the saphenous nerve in the femoral triangle.

In order to obtain reflex vasomotor effects, various nerves were stimulated. In the dogs, careful dissection of the vago-sympathetic trunk usually revealed a bundle of fibers, somewhat greyish pink in color, which had predominantly depressor effects (suggested by Dr. Stanley Nowak). In other experiments the left vagus and depressor, the nerve of Hering, or the median were employed. Stimulating current was supplied by a Harvard Inductorium with 3 or $4\frac{1}{2}$ volts in the primary circuit.

Since the dorsal root dilators have been found to be distributed primarily to skin areas, plethysmographs were designed to record changes in volume of the hind paws of the animals, as this region offers the greatest skin-muscle ratio of the limbs. Glass cylinders which just allowed the foot to enter were used and sealed around the foot with heavy vaseline well rubbed into the hair. In two acute experiments, performed on cats, muscle volume tracings were obtained from a conical metal plethysmograph, which was buried under the skin of the thigh. The chambers were connected by heavy rubber tubing to small segment capsules carrying a mirror at one side for optical recording. Calibration of each system was effected through a permanent side-arm connection with a straight 1 cc. pipette partially filled with mercury and fitted at the lower end with a closed rubber tube. Compression of the tube by a screw clamp, by raising the level of the mercury in the glass, had the effect of injecting a known volume of air into the plethysmograph system. This arrangement also provided a ready means of testing the recording system for air tightness. At the beginning of all experiments, and several times throughout their course, the plethysmographs were deflected to the limit of the recording scale by the introduction of air and observed for several minutes. Only if this test showed the plethysmograph seals to be tight were the records considered valid.

Since a rubber membrane served as the recording device, the measurements were not strictly volumetric, but none of the volume changes ob-

served involved a pressure change of more than 1.5 cm. of water. The slight theoretical disadvantage entailed was more than compensated for by the fact that the system was virtually inertialess when compared with volumetric recorders of comparable convenience. The sensitivity with a recording distance of 1 m. was such that 0.001 cc. change in volume equalled approximately 1 mm. on the record, and that a measurable change of paw volume showed readily with each heart beat. Active changes of caliber in the paw vessels could thus be estimated not only by modifications in the volume of the paw but also by changes in the pulse amplitudes. Passive changes of paw volume, especially those due to changes in venous pressure, gave little or no change in the pulse amplitude. Blood pressure was recorded optically from the carotid artery by a membrane manometer in the usual way.

Fig. 1   Fig. 2

Fig. 1. Negative effect in dog. Dial anesthesia, acute lumbar sympathectomy, evisceration, adrenals excluded, both vagi cut. Faradic stimulus during signal to left depressor nerve centrally, coil 7.5 cm. Upper tracing intact paw, lower tracing totally denervated paw, broad tracing blood pressure. Time in seconds in this and succeeding figures. *BP:* blood pressure.

Fig. 2. Negative effect in cat. Dial anesthesia, chronic bilateral lumbar sympathectomy (3 weeks' duration). Left vagus stimulated centrally, coil 10.5 cm. Reading downwards: blood pressure, sympathectomized paw, acutely denervated paw.

RESULTS. The experiments failed to demonstrate any changes in paw volume which could be attributed to reflex stimulation of the dorsal root dilators. The usual result of stimulating either depressor or carotid sinus nerves in the animals with lumbar sympathectomy was a fall in blood pressure (slight in the eviscerated preparations) accompanied by a decline in volume of the paws. The shrinking of the totally denervated side was, within the limits of the method, comparable to that on the side with sympathectomy only (figs. 1 and 2). This was true in spite of the fact that in many of the experiments, of which figure 1 is typical, the fall of blood pressure on depressor stimulation was very small owing to the exclusion of the splanchnic area from the circulation. Passive effects were thus minimal as shown by the slight fall in the volume of the denervated paw. In contrast with these negative effects, it was easy to demonstrate the well-known reflex inhibition of the vasomotor tone in paws with intact

sympathetic supply, even in the face of much greater falls of blood pressure and shrinking of the denervated control paw.

Passive effects only were observed in the two experiments employing a muscle plethysmograph on an acutely sympathectomized thigh (cf. Jarisch, 1925).

An objection may be raised to the experiments in which the sympathetic chains were removed at the time of the experiment. It might be said that the vessels acutely deprived of their vasocontrictors would be maximally dilated and thus unable to respond to a reflex activation of the dorsal roots. It is well known (Bayliss, 1902), however, that such vessels do respond to direct stimulation of dorsal roots, a fact which was confirmed in the only two experiments of this sort attempted in this series (cats).

Rarely in the cats (3 out of 16) and more commonly in the dogs (4 out of 6) increases in the volume of the sympathectomized paw were encountered. At some stage in the experiment, the usual passive fall in paw volume con-
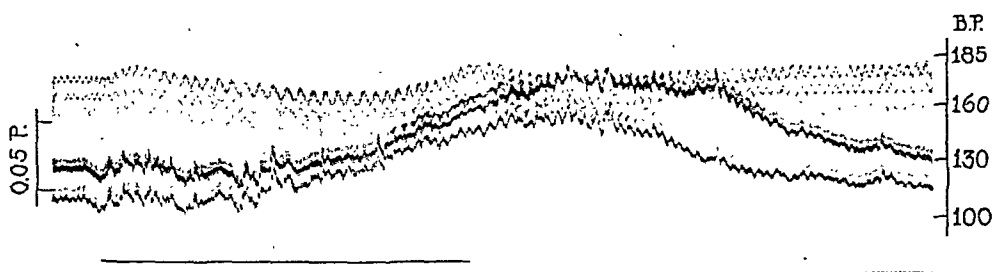

Fig. 3. False-positive effect in dog. Urethane anesthesia, acute lumbar sympathectomy, evisceration, both vagi cut. Depressor stimulation coil 10 cm. Reading downwards: blood pressure, sympathectomized paw, denervated paw. *P:* plethysmograph volume change in cubic centimeter.

sequent to depressor stimulation gave way to a rise in volume. This rise, however, generally took place in *both* the sympathectomized and the totally denervated paw. The increase in volume usually began about 20 to 30 seconds after the onset of stimulation, and continued until 10 to 20 seconds after stimulation ceased when a slow decline to the previous baseline set it. Figure 3 illustrates the most striking instance. The effect was inconstant, but in the dogs was repeatable to a greater or less degree at least three or four times during the experiment only to disappear without observable cause. The time relations suggest a humoral origin, but it occurred in several preparations in which the adrenals were ligated.

Changes in rate and depth of respiration brought about by depressor or carotid sinus stimulation could not be definitely correlated with the increases in paw volume, since the latter occurred during both increases and decreases in respiratory activity, and in one animal with bilateral pneumothorax and artificial respiration.

DISCUSSION. Bayliss and others have pointed to the occasional increase

in volume of sympathectomized limbs seen during depressor stimulation as evidence of true reflex activation of dorsal root dilators. The experiments presented here show that when such changes take place, entirely similar changes may occur simultaneously in completely denervated areas, and therefore seriously impair the cogency of the existing plethysmographic evidence as applied to this problem. As was pointed out in the introduction to this paper, other direct methods of investigation have almost without exception yielded negative results. The only other serious support for true reflex activation of dorsal root dilators is to be found in the indirect evidence educed from the reflex changes of blood pressure in sympathectomized animals (Freeman and Rosenblueth, 1931; Rosenblueth and Cannon, 1934; Pinkston, Partington and Rosenblueth, 1936). This puzzling phenomenon may, however, find an explanation on other grounds, although attempts to do so have not so far been satisfactory (cf. Brown, 1940).

Various theoretical considerations may be mentioned which militate against the view that the dorsal root dilators participate in reflex adjustments of blood pressure by the circulatory proprioceptors. The dilator fibers in the dorsal roots have been shown to belong to the slowly conducting "C" group (Hinsey and Gasser, 1930). Anatomically (Ranson and Billingsley, 1916) and physiologically (Clark, Hughes and Gasser, 1935; Heinbecker and Bishop, 1935) this component of the dorsal roots has been shown to be concerned with pain sensation, and there are no grounds for believing that a specific portion of it is reserved for a vasomotor function. In fact the classical diagram of the axon reflex assumed the identity of the dorsal root dilators with sensory fibers. If this assumption is correct, it is hard to see how their central activation could take place without alteration of the normal synaptic activity involved in the transmission of pain.

The researches of Lewis (1927) and others have shown that the dorsal root dilators are principally concerned with localized reactions in response to damaging stimuli (i.e., inflammation). In accordance with this, responses evoked by direct stimulation have a relatively long latency and a long duration, up to fifteen minutes. Bayliss, observing this long duration, dismissed it as due to congestion from the pressure of the plethysmograph cuff, but later observations with other techniques have established the phenomenon (Langley, 1923; Wybauw, 1936; Lewis and Marvin, 1927).

The properties listed above would apparently render the dorsal root dilators unsuited for the purpose of making reflex adjustments of the blood pressure in response to the ever changing stimuli supplied by the receptors in the aortic arch and carotid sinus.

It is unfortunate, though unimportant for the present argument, that

we are unable definitely to state the origin of the dilator reactions observed in totally denervated paws. The caliber of blood vessels is of course influenced by many non-nervous factors one or more of which may account for the results obtained in this and other investigations. Whatever the nature of these effects, it seems clear that a dilatation observed in a sympathectomized hindlimb during a fall of blood pressure is not sufficient evidence that an extrasympathetic nervous control is in operation. We conclude that the theory that the vasodilator fibers in the dorsal roots can be activated reflexly from the central nervous system lacks any adequate direct support.

SUMMARY

Simultaneous recording of the volume of a sympathectomized and of a completely denervated paw during reflex depressor stimulation revealed no differences between the reactions of the blood vessels of the two sides, although both increases and decreases of paw volume were produced.

It is concluded that the existing evidence does not favor the concept of reflex activation of the dorsal root dilators.

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THE ASCORBIC ACID, PHOSPHATASE AND CALCIUM CONTENT OF THE BLOOD OF GUINEA PIGS WITH VARYING DEGREES OF SCURVY[1]

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One of the characteristic symptoms of scurvy is the lack of intercellular material in the bones and the ease with which the bones of guinea pigs are fractured.   Observations of this condition aroused our interest in the possible relation between ascorbic acid and calcium content of the blood and the phosphatase activity of the serum.   This problem therefore has been investigated using groups of guinea pigs receiving a high ascorbic acid intake, a subnormal and a very low ascorbic acid intake.

METHODS. *Experimental animals.*   Healthy guinea pigs, six to eight weeks of age, and weighing 280 to 300 grams were fed a vitamin C-free basal diet supplemented with different amounts of ascorbic acid.   The basal diet consisted of rolled oats 36 per cent, bran 18 per cent, heated skim milk powder 30 per cent, dried yeast 5 per cent, hydrogenated fat (Crisco) 8 per cent, cod liver oil 2 per cent, sodium chloride 1 per cent. The experimental period lasted 34 days and at the end of that time the animals were destroyed and scored for scurvy by the method of Sherman, LaMer and Campbell (1922).

Determination of ascorbic acid.   The ascorbic acid content of the oxalated blood plasma was determined by the micro-method of Farmer and Abt (1936) with an accuracy of ±0.08 mgm. per 100 ml. of plasma.

Determination of serum calcium.   Serum calcium was determined by the Tisdall (1923) modification of the Kramer and Tisdall (1921) method, but with the exception that the wash solution was added to the surface of the precipitate and not mixed with it.   By this method 98 per cent of added calcium was recovered.

Determination of serum phosphatase.   Phosphatase activity was determined by the King and Delory (1937) micro-method and is expressed

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as units per 100 ml. of serum. Each unit is equal to the milligrams of phenol liberated from a di-sodium phenyl phosphate substrate in 15 minutes at 37°C. and pH 9.0. Color comparisons were made in a Duboscq colorimeter. The micro-method was chosen because of the small amount of serum required. According to King and Delory, the results obtained by this method are quantitatively equal to those obtained by the macro-method of King (1934). Brown (1936) in a comparative study of the different methods of phosphatase determination found that the method of King (1934) gave results consistent with those obtained by the Bodansky method (1932).

Blood samples. For the ascorbic acid determinations, blood was taken from the heart of the etherized animal using a syringe which had previously been rinsed with a 2 per cent solution of lithium oxalate. For the serum calcium and phosphatase determinations the throat of the animal was severed and the body drained of blood. Ether was reported by Bowman and Muntwyler (1937) to cause an increase in the amount of ascorbic acid excreted in the urine, and Ecker (1938) reported that there was a rise in serum ascorbic acid after ether anesthesia. Since ether might possibly have caused a change in the level of the blood constituents being studied a preliminary investigation was made with blood samples taken without ether and after 15 and 45 minutes under ether anesthesia. Plasma ascorbic acid and serum phosphatase were determined under these conditions, but insufficient blood was obtainable from each animal for similar studies on calcium content.

Plasma ascorbic acid values of blood samples taken from four animals without ether were 0.41, 0.30, 1.03 and 0.46 mgm. per 100 ml. whereas after seven minutes under ether anesthesia the plasma values were 0.44, 0.30, 0.97 and 0.44 mgm. per 100 ml. respectively. There was a rise in the plasma ascorbic acid content after 45 minutes of ether the respective values being 0.56, 0.36, 1.17 and 0.58 mgm. per 100 ml. Since there was no appreciable change in plasma ascorbic acid in seven minutes of ether, all blood values reported in this paper were taken within seven minutes of the time when ether was first given.

Determination of serum phosphatase activity of blood samples from three animals showed the following values: without ether, 9.2, 14.4, 12.0 units per 100 ml.; under ether for 15 minutes 9.5, 14.1, 11.5 units per 100 ml. and under ether for 45 minutes 12.3, 12.4 and 15.7 units per 100 ml. There was no change in phosphatase activity after 15 minutes of ether and therefore all blood samples for serum phosphatase determinations were collected within 15 minutes after administration of ether.

Blood samples for calcium determinations were taken at the same time as those for phosphatase.

Series I. Blood plasma ascorbic acid in relation to ascorbic acid intake.

The ascorbic acid content of the blood plasma of monkeys and man has been shown to be dependent upon the dietary intake of the vitamin (Farmer and Abt, 1935; Greenberg, Rinehart and Phatak 1937). Few data have been reported for blood plasma of guinea pigs and the available evidence does not indicate what is the ascorbic acid content of the blood of so-called normal animals or whether the ascorbic acid content is influenced by the diet. In order to clarify this question, blood determinations were made on all available guinea pigs in the laboratory; these included animals which had received 8 mgm. of crystalline ascorbic acid[3] daily for 34 days, and animals which had received 2 mgm. of ascorbic acid daily from lemon juice or from the crystalline form of the vitamin for the same period of time. Another group of animals being used for vitamin C assay of certain fruits received basal diet only for 13 days followed by daily fruit supplements which supplied approximately 0.5 mgm. of ascorbic acid daily for 21 days. A group of negative controls was maintained on the vitamin-C-free basal diet until they showed signs of severe scurvy; blood samples were then taken and the animals were destroyed.

The mean plasma ascorbic acid values for the different groups of animals are summarized in table 1. The negative controls showed no measurable amount of ascorbic acid in the blood plasma. Those animals which had been made scorbutic and then fed various fruit supplements supplying approximately 0.5 mgm. ascorbic acid daily had scurvy scores ranging from 0 to 11 and the blood plasma ascorbic acid content was 0.16 mgm. per 100 ml. with a standard deviation of ±0.07. The mean value for plasma ascorbic acid of animals receiving 2.0 mgm. of ascorbic acid daily was 0.22 mgm. per 100 ml. with a standard deviation of ±0.03. Statistical treatment of the data of these two groups as described by Snedecor (1938) for determination of t gives a t value of 4.962 which shows that the difference between the two groups is significant.

Guinea pigs which received 8 mgm. of ascorbic acid daily had a mean plasma content of 0.54 mgm. per 100 ml. with a standard deviation of ±0.11; the t value of 9.119 shows this to be a highly significant increase in blood ascorbic acid over the group receiving 2.0 mgm. daily. Thus the ascorbic acid content of the blood plasma of guinea pigs is seen to be dependent on the intake.

Series II. Ascorbic acid, calcium, and phosphatase content of blood of guinea pigs with varying degrees of scurvy. In this series three groups of animals were used and were comparable in every way except for the amount of vitamin C fed and the consequent degree of scurvy. In group I there were 8 animals which had received 8 mgm. ascorbic acid daily throughout

[3] We are indebted to Merck and Company, Rahway, New Jersey for a generous supply of ascorbic acid.

the experimental period; these animals appeared to be healthy and increased rapidly in weight during the period. There were 21 animals in group II and on autopsy all showed mild scurvy with scores ranging from one to eight. In group III there were 10 animals all of which showed severe scurvy with scores of 9 to 16. The ascorbic acid content of the blood plasma, and the phosphatase and calcium content of the serum of each animal were determined with the exception of six animals where the blood supply was inadequate for the calcium determination. The data are summarized in table 2. No measurable amount of ascorbic acid was found in the blood plasma of animals with severe scurvy; those with mild scurvy had an average of 0.20 mgm. ±0.062[4] per 100 ml. and the normal animals averaged 0.57 mgm. ±0.105 per 100 ml. The difference between the two latter groups is significant, t value being 9.38.

TABLE 1

Ascorbic acid content of blood plasma of guinea pigs with different intakes of ascorbic acid

ASCORBIC ACID INTAKE DAILY	NUMBER OF ANIMALS		SCURVY SCORE	ASCORBIC ACID PER 100 ML. PLASMA	STANDARD DEVIATION	t VALUE
	Males	Fe-males				
				mgm.		
8 mgm. for 34 days..............	6	4	0	0.54	0.11	9.119
2 mgm. for 34 days..............	6	6	0	0.22	0.03	
Basal diet only for 13 days then 0.5 mgm. for 21 days..........	40	33	0–11	0.16	0.07	4.962

In the normal animals the mean phosphatase value in units per 100 ml. of serum was 17.0. Mild scurvy caused a slight rise in phosphatase activity to 21.0 units and the t value for the mean difference between these two groups was 2.29 which is barely significant. The animals with severe scurvy showed a marked lowering in phosphatase to 6.8 units. The t value of the mean difference in phosphatase value between this group of animals with severe scurvy and the normal animals was 5.49; the t test also showed that the difference between the means for the group with mild scurvy and the group with severe scurvy was significant, the value being 9.47.

The mean serum calcium content was approximately the same for all three groups being 13.1, 13.9 and 13.0 mgm. per 100 ml. of serum.

A comparison was also made of the blood constituents for each sex but in some groups insufficient numbers of animals had been used to draw any conclusions. There appeared to be no difference in the ascorbic acid

[4] Standard deviation.

TABLE 2

Ascorbic acid, phosphatase and calcium content of blood of scorbutic and non-scorbutic guinea pigs

DEGREE OF SCURVY	ANIMAL NO.	SEX	SCURVY SCORE	PLASMA ASCORBIC ACID	SERUM PHOSPHA-TASE	SERUM CALCIUM
				mgm. per 100 ml.	units per 100 ml.	mgm. per 100 ml.
None (8 mgm. ascorbic acid daily)	1	M	0	0.69	24.9	12.6
	2	M	0	0.72	20.2	11.5
	3	M	0	0.57	15.2	15.3
	4	F	0	0.55	17.6	14.1
	5	M	0	0.53	13.8	14.6
	6	M	0	0.62	13.6	14.4
	7	M	0	0.44	17.8	11.7
	8	F	0	0.44	12.6	10.8
Mean..........................				0.57	17.0	13.1
Mild (score 1–8)	9	F	2	0.21	22.4	
	10	F	5	0.17	20.5	9.8
	11	M	1	0.21	17.8	15.1
	12	M	3	0.23	21.6	14.9
	13	M	3	0.16	17.4	13.7
	14	F	5	0.16	17.0	13.3
	15	M	1	0.34	16.7	13.4
	16	M	1	0.26	15.8	12.6
	17	M	1	0.21	25.6	12.4
	18	M	3	0.30	29.8	12.6
	19	M	1	0.21	24.0	15.0
	20	M	3	0.22	27.4	14.0
	21	F	1	0.23	16.3	10.2
	22	F	3	0.10	16.8	11.4
	23	M	6	0.10	23.6	11.9
	24	F	4	0.17	17.1	12.9
	25	M	4	0.25	26.1	12.3
	26	F	2	0.24	20.2	13.8
	27	F	4	0.26	14.8	
	28	M	7	0.12	27.0	15.2
	29	F	1	0.15	24.1	
Mean..........................				0.20	21.0	13.0
Severe (score 9–16)	30	F	9	0	11.6	15.3
	31	F	11	0	11.4	14.7
	32	F	9	0	8.3	
	33	F	12	0	4.6	11.9
	34	F	14	0	3.4	12.4
	35	F	13	0	5.2	
	36	M	15	0	4.2	14.5
	37	M	10	0	6.6	15.5
	38	M	14	0	9.5	
	39	M	15	0	2.7	13.0
Mean..........................				0	6.8	13.9

content of the blood plasma of males and females.   In the group receiving 2 mgm. of ascorbic acid per 100 mgm. plasma the average for six males was 0.24 mgm. and for six females was 0.21 mgm.   In the group receiving 0.5 mgm. of ascorbic acid the average plasma content for 40 males was 0.15 mgm. and for 33 females it was 0.17 mgm.

Similarly the calcium content of the blood of the two sexes was comparable.   In the group with scurvy scores ranging from 1 to 8 the average calcium value for 12 males was 13.6 mgm. per 100 ml. and for 6 females was 11.9 mgm.   In the group with scurvy scores of 9 to 16 the average for three males was 14.3 mgm. and for four females 13.6 mgm. per 100 ml.

There was considerable variation in the phosphatase values within each group; for those having scurvy scores of 1 to 8, a group of 9 females had an average of 18.8 phosphatase units per 100 ml. and a group of 12 males 22.7 units.

Series III.   Ascorbic acid, calcium, and phosphatase content of blood of animals with restricted food intake.   Guinea pigs suffering from scurvy show loss of appetite and lowered food intake, and it is possible that the lowered food consumption of the scorbutic animals studied in series I and II might have been an influencing factor on the levels of the blood constituents.   In this series, therefore, the paired-feeding method was used so that the food intake for each pair of animals was comparable.   Six pairs of guinea pigs were used, one animal of each pair received basal diet only and the other received 8 mgm. of ascorbic acid daily in addition to the amount of basal diet equivalent to that which had been consumed by the corresponding pair-mate on the previous day.   The experimental period was 34 days except for two animals on basal diet only that lived 26 and 28 days and the pair-mates were killed on the corresponding day.

The ascorbic acid, calcium, and phosphatase values are summarized in table 3.   The scorbutic animals had scurvy scores ranging from 9 to 15 and no measurable amount of ascorbic acid in the plasma.   Pair-mates receiving 8 mgm. of ascorbic acid daily had plasma values ranging from 0.31 mgm. to 0.41 mgm. per 100 ml. which is considerably lower than the ascorbic acid content of the blood plasma of the animals in series II with the same ascorbic acid intake but unrestricted food consumption.

The serum phosphatase activity of the animals receiving 8 mgm. of ascorbic acid daily was markedly greater than that of the scorbutic pair-mates but was lower than the mean value for animals in series II that also received 8 mgm. ascorbic acid daily and unrestricted food intake.

The serum calcium levels for the pair-mates in this series were comparable and were similar to the animals in series II.

In table 3 the weight gains for the pairmates are also shown.   Although the average daily food intake was approximately the same for each pair

of animals those receiving ascorbic acid daily showed much greater gains in weight. McHenry, Reedman and Sheppard (1938) have reported similar findings and attribute the weight difference to the diminished retention of water in the bodies of animals which lack ascorbic acid (Sheppard, McHenry 1939).

· Discussion. The data reported here clearly demonstrate that the ascorbic acid content of the blood of guinea pigs is dependent on the dietary intake of this substance and is not caused by the lowered food intake which accompanies the development of severe scurvy. These findings are in

TABLE 3

Ascorbic acid, calcium and phosphatase content of blood of guinea pigs in paired feeding study

GUINEA PIG NUMBER	SEX	ASCORBIC ACID INTAKE PER DAY	BODY WEIGHT				AVERAGE DAILY FOOD INTAKE	EXPERIMENTAL PERIOD	SCURVY SCORE	PLASMA A.-A.	SERUM PHOSPHATASE	SERUM CALCIUM
			Initial	Maximum	Final	Total gain						
	·	mgm.	grams	grams	grams	grams	grams	days		mgm. per 100 ml.	units per 100 ml.	mgm. per 100 ml.
1	M	0.0	300	415	319	19	15	34	13	0	5.2	
2	M	8.0	291	390	360	69	15	34	0	0.40	15.8	13.0
3	F	0.0	308	330	235	−73	12	28	14	0	3.4	12.4
4	F	8.0	302	316	290	−12	10	28	0	0.32	5.0	13.4
5	F	0.0	312	316	215	−97	·11	26	12	0	4.6	11.9
6	F	8.0	305	331	312	7	11·	26	0	0.41	11.7	14.8
·7	M	0.0	·304	435	367	63	20	34	9	0	6.6	15.5
8	M	8.0	313	470	440	127	17	34	0	0.40	15.2	12.8
9	M	0.0	302	302	197	−105	·13	34	15	0	2.7	13.0
10	M	8.0	305	385	360	55	13	34	0	0.34	·6.6	15.0
11	F	0.0	300	300	177	−123	10	34	14	0	9.5	
12	F	8.0	296	335	272	−24	10	34	0	0.31	11.4	13.8

agreement with those of other workers who have reported on the blood ascorbic acid of man and of monkeys in relation to the diet. Ecker and co-workers (1938) have reported values of 1.04 to 2.13 mgm. per 100 ml. of blood serum for guinea pigs fed green food for eight days which is considerably higher than the values obtained in this study for animals fed 8 mgm. ascorbic acid daily.

Serum phosphatase activity has been reported to be affected by abnormal conditions such as jaundice and various bone diseases as well as scurvy. Smith and Maizels (1932) have reported that there was a definite

increase in serum phosphatase activity in rickets in young children and this serum phosphatase rise has been recommended as a valuable index of early cases of rickets (Barnes and Carpenter, 1937). The bone changes taking place in scurvy are apparently caused by a lack of formation of intercellular substances (Wolbach, 1926) and a cessation of ossification and growth. This may account for the low phosphatase values reported in cases of scurvy in children (Smith and Maizels, 1932). Scoz and co-workers (1937) found first an increase in phosphatase activity and then a decrease during the progress of scurvy in guinea pigs. The data reported here show that animals with mild scurvy as indicated by scurvy scores of one to eight, showed some increase in serum phosphatase activity but statistical analysis shows that the increase is probably not significant. However, in animals with severe scurvy the serum phosphatase activity was significantly lower than that of healthy animals. Paired feeding experiments demonstrated that the degree of scurvy and not the restricted food intake was the cause of lowered phosphatase activity of the serum since the pair mates with similar food intake but adequate ascorbic acid had markedly higher phosphatase values.

Our findings with regard to the calcium content of the blood serum are in agreement with those of Goettsch and Key (1928) and Humphrey and Zilva (1931) who found a wide range in the serum calcium but no indication that these changes paralleled scurvy. Lowered food intake in the later stages of scurvy had no effect on the calcium level of the blood.

SUMMARY

The ascorbic acid, calcium, and phosphatase of the blood of guinea pigs receiving varying amounts of ascorbic acid as supplements to the basal ration were studied. The ascorbic acid content of the blood was found to be negligible in scurvy and to increase as the dietary intake was increased. A daily supplement of 8 mgm. ascorbic acid during the experimental period gave a mean blood value of 0.54 mgm. per 100 ml. of plasma; the corresponding value was 0.22 mgm. per 100 ml. when the daily intake was 2 mgm. ascorbic acid.

The phosphatase activity of the serum was increased slightly in mild scurvy and decreased markedly in cases of severe scurvy.

Serum calcium values remained unchanged in scorbutic animals.

Differences observed in phosphatase values and ascorbic acid content of the blood were not attributable to sex or to the amount of basal diet eaten, and were unchanged during 15 minutes of ether anesthesia.

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A HISTAMINE-LIKE SUBSTANCE IN THE POST-PARTUM RABBIT UTERUS[1]

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Among the characteristic and consistent changes in the rabbit uterus during the final stages of pregnancy, three may be especially important in the sequence of events leading to parturition. Two to three days before delivery the following changes can be observed: 1, increased color, of vascular origin, approaching a bluish-red on the day of delivery (Markee and French, 1934); 2, increased volume of the entire uterus reaching a maximum on the day of delivery, then slowly receding for 6 to 8 days post-partum (Markee and Andersen, 1934), and 3, increased amounts of intercellular fluid, or edema, reaching a peak on the day of delivery, then gradually receding for 6 to 8 days after delivery (Krichesky, unpublished).

In attempting to determine whether a full term or immediately post-partum, edematous uterus contains a local factor capable of inducing edema in a non-pregnant uterus, it was found (Krichesky and Pollock, 1940) that a crude saline extract of rabbit uterus, taken within a few hours after delivery, is extremely toxic to non-pregnant animals when injected intravenously in relatively small doses. This study was undertaken to confirm these previous findings and to determine, if possible, the nature of the toxic substance in extracts of full term edematous uteri.

MATERIALS AND METHODS. A total of 71 rabbits were used in this study; of these 54 were non-pregnant females, 12 were females in late stages of pregnancy or a few days post-partum, and 5 were males.

Four experiments were carried out: 1, to determine the toxicity of saline extracts of post-partum rabbit uteri; 2, to ascertain the toxicity of non-pregnant uteri and of skeletal muscle from post-partum rabbits; 3, to establish whether or not blood serum or whole blood from animals in late stages of pregnancy and immediately post-partum is toxic, and 4, to arrive at the nature of the toxic factor in post-partum uterine extracts.

In the first experiment all extracts were prepared as follows: Uteri were excised within a few hours after delivery, weighed, and ground to a mash with washed sand. A volume of saline equal to half the weight in grams of the fresh tissue was added. The mixture was shaken intermittently for

30 minutes, then centrifuged. The supernatant fluid was collected and the residue re-extracted. The supernatant fluids from the two extractions were combined and saline added until 1 cc. of the final solution represented 0.5 gram of fresh tissue. Merthiolate (1:50,000) was added and the extracts stored in a refrigerator. All extracts were used within four days of their preparation.

In the second experiment extracts of non-pregnant uteri and of muscle were prepared in the manner described. Abdominal muscles and portions of the thigh muscles were taken from post-partum animals used in experiment 1.

For experiment, blood was collected from the marginal ear vein of donor rabbits. Serum alone was used in some instances and whole blood in others.

It soon became evident that the toxic factor in post-partum uterine extracts was histamine-like in its action. For this reason, in the fourth experiment attempts were made to inactivate the toxic substance by using the enzyme histaminase (Torantil 360).[2] Histaminase in various proportions was added to both histamine and to toxic uterine extracts, the pH adjusted to 7.2 and the mixtures incubated for 24 hours at 37.5°C., then filtered, and the filtrate injected.

All animals were injected intravenously with a maximum single dose of 10 cc. Where larger doses were given they were administered by an additional maximum of 10 cc. on each successive day.

RESULTS. *Experiment 1. Toxicity of post-partum uterine extracts.* A total of 9 extracts were prepared from rabbit uteri taken between 2 and 12 hours after delivery, 10 within 7 hours and only two as long as 10 and 12 hours post-partum. These were injected intravenously into a total of 23 non-pregnant animals. The average lethal dose was 5.6 cc. representing 2.8 grams of fresh tissue. The potency of the individual extracts varied considerably, but in all instances the recipients succumbed within two minutes after injection. The least toxic extract required an average dose of 14.5 cc. to cause death. In the preparation of this extract the excised uterus was allowed to stand in the refrigerator for approximately 34 hours before extraction and it is possible that the toxic factor was partially destroyed or inactivated during that time. Only one extract was lethal in doses as small as 0.5 cc.

Young and old animals of both sexes proved equally susceptible. Although complete data were not obtained it appeared that the lethal dose was approximately proportional to body weight.

In addition to the animals mentioned above, three rabbits were injected

[2] Histaminase (Torantil 360) was generously supplied by the Department of Medical Research, Winthrop Chemical Company, Inc.

intraperitoneally. When given by this route the extracts also proved fatal, although a larger dose was required and a longer time elapsed before death.

Since there are large necrotic areas and masses of autolyzed cells in the post-partum uterus, the possibility existed that animals possessing such a uterus might be less susceptible to toxic extracts than non-pregnant animals. Toxic uterine extracts were injected into the following animals: one animal one day pre-partum, two animals on the day of delivery and two animals one day post-partum. These five animals died within two minutes of administration of approximately the same quantity producing death in non-pregnant animals. Thus the presence of an edematous uterus *in situ*, containing the toxic factor, does not influence the susceptibility of the animal to extracts of known toxicity.

Experiment 2. Effects of extracts of non-pregnant uterus and of muscle. In this experiment extracts of non-pregnant uteri and of muscles were prepared in the manner described for post-partum uteri, each cubic centimeter representing 0.5 gram fresh tissue.

Five extracts of non-pregnant uteri were injected intravenously into a total of 8 animals. The average dose was 26.2 cc. representing 13.1 grams of fresh tissue. Four of these extracts proved harmless in doses ranging from 15 to 37.5 cc., while one extract proved toxic in a dose of 20 cc. All animals received large doses and even in the only case of toxicity in an extract of non-pregnant uterus the lethal dose was almost five times greater than the average dose employed in experiment 1. These results indicate that the toxic factor present in post-partum uterine extracts is either absent or present in only low concentrations in non-pregnant uteri.

As a further control for experiment 1, four extracts were prepared from skeletal muscle taken from the same animals providing uterine material for extracts used in experiment 1. Six non-pregnant rabbits were given an average dose of 41.7 cc. of this material, representing 20.8 grams of fresh tissue. In no case did the muscle extracts prove fatal, indicating that if the toxic factor was present its concentration was too low to produce symptoms in the quantities used.

Experiment 3. Effects of blood serum and of whole blood from gravid and post-partum rabbits on non-pregnant animals. During late stages of pregnancy and immediately post-partum the rabbit uterus contains a larger volume of blood than does the non-pregnant uterus or muscle, consequently the possibility existed that the toxic factor in post-partum uterine extracts was present in the blood rather than in the tissues. Blood was therefore collected from the marginal ear vein of both gravid and post-partum animals. Blood serum alone was injected into 5 rabbits in quantities as high as 20 cc. Whole blood was given to two animals in doses of 16 cc. All animals survived this treatment, indicating that neither blood serum

nor whole blood, in the amount used, contained a sufficiently high concentration of the toxic factor to be lethal.

Experiment 4. Inactivation of histamine solutions and toxic uterine extracts by incubation with histaminase. All animals that succumbed from an injection of toxic uterine extracts exhibited symptoms preceding death strikingly similar to those described for fatal anaphylaxis. There was a blanching of the eye and ear, extreme respiratory difficulty, accompanied by jerking motions of the head and body. On autopsy the lungs were characteristically distended and the right chambers of the heart engorged. The phenomenon was not considered an anaphylaxis, however, since none of the animals had been previously sensitized.

Since several investigators reported a possible relation between histamine and anaphylaxis (Dale and Laidlaw, 1910; Bally, 1929; Dragstedt and Gebauer-Fuelnegg, 1932; Code, 1939 and others), it seemed possible that the toxic factor in post-partum uterine extracts might be histamine or histamine-like.

Four rabbits were injected with a histamine solution containing 1.0 mgm. per cubic centimeter. Two of these were given a total of 0.5 cc., or 0.5 mgm., and two a total of 1.0 cc., or 1.0 mgm. The smaller dose produced severe shock from which both animals recovered, while the larger dose killed the animals in less than two minutes and reproduced accurately the symptoms and pathological changes following administration of toxic uterine extracts. This provided further evidence that the toxic factor might be histamine or a histamine-like substance.

Best and McHenry (1930, 1931) reported the isolation of an enzyme, histaminase, which destroyed the activity of histamine when incubated with it. The enzyme itself is not toxic even when given in relatively high concentrations (Barlow, unpublished). Three rabbits were given 3, 5 and 10 units respectively of histaminase intravenously and exhibited no ill effects. In accord with the work of Best and McHenry (1930, 1931) two rabbits given 4 and 5 times the lethal dose of histamine previously incubated with histaminase also exhibited no symptoms, indicating that the amine had been inactivated.

Since it appeared that the toxic factor in uterine extracts might be histamine, extracts of known toxicity were incubated with histaminase, then tested for activity. To 40 cc. of one extract 25 units of histaminase were added, the pH adjusted and the mixture incubated for 24 hours at 37.5°C. and then filtered. The filtrate was given to 4 animals in doses of 9 cc. (9 times the lethal dose of this extract before inactivation). None of these animals succumbed and only one exhibited symptoms of shock. In another instance, 50 cc. of an extract were incubated with 20 units of histaminase and the filtrate given to four animals in doses of 12 cc. Although this was four times the lethal dose of this extract before treatment

with histaminase, it failed to produce any of the characteristic symptoms of toxicity.

The addition of 8 units of histaminase to 12.0 cc. of another extract but without subsequent incubation, had no effect on toxicity when tested on two animals. The incubation of an extract of known toxicity but without added histaminase also failed to alter the toxicity of the material. These results indicate that the toxic factor in extracts of the post-partum rabbit uterus is inactivated by the action of histaminase and consequently it may be assumed that the toxic factor is histamine or a histamine-like substance.

DISCUSSION. It is unusual that saline extracts of normal tissue are toxic to animals of the same species. However, such extracts of the post-partum rabbit uterus, taken within 12 hours after delivery, are extremely so. Furthermore, the absence of toxicity from similar extracts of the non-pregnant uterus indicates that the toxic material is associated with a particular physiological state.

Our results indicate that the toxic factor is either histamine or a substance histamine-like in its physiological properties and chemical structure. Reference has been made to the reported relation between histamine and anaphylaxis. Symptoms preceding death and pathological changes after death, following administration of toxic uterine extracts, are accurately reproduced by lethal quantities of histamine.

A more specific test is the inactivation of the toxic material by incubation with histaminase. By this means the activity is destroyed and some structural similarity to histamine is inferred, since it is suggested by Best and McHenry (1930) that histaminase acts on histamine by destruction of the imidazole ring.[3]

The presence of large amounts of a substance with histamine-like action may possibly explain some of the characteristic histological and physiological changes in the full term rabbit uterus. The intensity of color, of vascular origin, increases to a bluish-red on the day of delivery and then slowly diminishes (Markee and French, 1934). Observations by one of us (Krichesky, unpublished) indicate that the capillary bed and small venules of the endometrium are greatly dilated and filled with blood cells beginning two days pre-partum until two days post-partum. This can be explained by a constriction of the veins with a consequent dilatation in venules and capillaries, producing stasis. Capillary dilatation and venous constriction after histamine has been reported by several investigators (Feldberg, 1927;

[3] In addition, extraction of histamine by the method of Barsoum and Gaddum, (1935) was carried out on one extract and the resulting substance, even in dilute solutions, produced contractions of the guinea pig ileum immersed in Tyrode's solution, providing further evidence that the toxic factor is histamine or histamine-like in its properties.

Ruhman, 1932; Sturm and Dauter, 1937 and others). By this action on the vascular system, it is possible that a substance with histamine-like properties may account for the increased intensity of color in the uterus and endometrium toward the end of pregnancy.

The presence of a substance with histamine-like activity may explain adequately both the growth in size of uterus and endometrium during the last two or three days of pregnancy (Markee and Andersen, 1934), and the accumulation of intercellular fluid (Krichesky, unpublished). The H-substance postulated by Lewis (1927) in the triple response of the skin to histamine or mechanical stimulation, suggests increased capillary permeability. It has been demonstrated also by Ravenna (1930), Kiuchi (1937), and others, that capillary permeability is increased by the administration of histamine. Consequently, the presence of large amounts of histamine, or a substance with histamine-like activity, because of its effect on capillary walls, may produce an edema resulting in an increase in the size of the organ.

The expulsion of the embryos at the end of pregnancy may be influenced also by the presence of a high concentration of a histamine-like substance in the full term rabbit uterus. Dale and Laidlaw (1910) recorded the contractions of the uterus in a pithed cat after administration of 1.0 mgm. histamine intravenously. *In vitro* experiments by tum Suden (1934) using rats, and Sugimoto (1937), using several different animals, obtained uterine contractions on addition of histamine. In view of the work of Barsoum and Gaddum (1935) who found that rabbit blood contains the highest histamine equivalent in the series of common laboratory animals studied, it is not surprising that Sugimoto found the rabbit uterus least sensitive in the series of animals he studied.

Bourne and Burn (1927), however, in some clinical studies, reported that although injections of 2 mgm. of histamine under the skin produced powerful but short lived contractions of the uterus, labor was not accelerated. They do not consider histamine a factor initiating termination of pregnancy because, in their experiments, uterine muscle activity ceased after the effect of histamine had worn off.

Little is known quantitatively or qualitatively of histamine in the full term human uterus, but in the rabbit, at least, it is difficult to conceive of the presence of large amounts of a histamine-like substance capable of exerting such a powerful effect on smooth muscles without its taking some part in the contractions of the uterus leading to parturition. In agreement with this view is the work of Danforth (1939) who reported that a high histaminase content of the human placenta is accompanied by weak uterine contractions whereas a low histaminase content of the placenta is accompanied by vigorous contractions. This suggests that in the case of high histaminase content, histamine or histamine-like substances are inactivated

resulting in weak uterine contractions, strong contractions being present only when the histaminase content is low.

In addition, Marcou and Atanasia-Vergu (1937) find that the concentration of blood histamine is lower in pregnant than in normal women and that the concentration falls very low, less than 0.05 gamma per cubic centimeter, during labor. They suggest that the histamine is taken up by other organs, but it is possible, in the rabbit at least, that it is taken up by the uterus itself, since a high concentration of a histamine-like substance is present in the rabbit uterus at that time. The mechanism of this decrease is unknown and Marcou and Atanasia-Vergu postulate that a low level of blood histamine, normally a vasodilator, may reduce post-partum hemorrhage. However, high concentrations of histamine are known to produce constriction of the arteries (Feldberg, 1927; Wilson, 1936 and Kiuchi, 1937). The possibility exists, therefore, that post-partum hemorrhage may be reduced not because of a low concentration of histamine in the blood but because of arterial constriction due to a high concentration of histamine or a histamine-like substance in the uterus at that time.

SUMMARY

1. Saline extracts of post-partum rabbit uteri, taken within 12 hours after delivery, contain a toxic substance lethal to non-pregnant animals.

2. With one exception, the toxic factor was not present in non-pregnant uteri or in skeletal muscle, blood serum, or whole blood of rabbits in late stages of pregnancy or one day post-partum.

3. Animals one day pre-partum to one day post-partum are no less susceptible to the toxic factor than are non-pregnant animals.

4. The toxic factor is probably histamine or a histamine-like substance. It resembles histamine in physiological properties and is readily inactivated by incubation with histaminase.

5. The presence of histamine or a histamine-like material in the full term and post-partum rabbit uterus is discussed in relation to events leading to parturition, i.e., color change, edema, muscle contractions and post-partum hemorrhage.

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THE IN VITRO SECRETION OF ACID BY THE GASTRIC MUCOSA OF THE FROG

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In 1930 Delrue (1) reported that the isolated gastric mucosa of the frog, when mounted properly in a dual-chambered bath, will secrete acid and will respond to the addition of drugs. The same author subsequently demonstrated (2) the effects of varying the ionic composition of the artificial medium on the *in vitro* production of acid. The theoretical importance of this approach to the study of the mechanism of acid formation by the gastric glands is apparent. However, to our knowledge, this work has never been confirmed, nor has the method been applied to the problem of acid formation.

METHODS. From a pithed frog the stomach, together with a short segment of the esophagus and duodenum, was removed and washed out with isotonic saline. An incision was made along the lesser curvature through the muscularis layer and the intact mucosal layer was dissected free. The resulting tube of mucosa was cut along the lesser curvature and spread out as a flat membrane over a hole, one centimeter in diameter, made in the center of a thin Monel metal plate 4 cm. square. Another similar metal plate was placed on top of the mucosa and the two were bolted together with the mucosa held firmly between them. This assembly was then mounted between two small glass chambers shaped like $CaCl_2$ tubes. Each chamber had a hole $\frac{1}{2}$ inch in diameter ground on one side. The chambers were clamped together with a modified burette clamp in such a way that the edges of the holes in the glass chambers were pressed in water-tight contact with rubber gaskets which had been cemented around the holes in the metal plates. The contents of the two chambers were thus separated only by the thin layer of the gastric mucosa.

One chamber with a capacity of 6 cc. was filled with 0.7 per cent NaCl solution through which air was bubbled; this solution bathed the natural secretory surface of the mucosa. The other chamber with a capacity of 15 cc. was filled with an artificial nutrient solution aerated with 95 per cent oxygen and 5 per cent carbon dioxide; this solution bathed the side of the mucosa which had originally been in contact with the muscularis.

The fluid level in both chambers extended above the upper edge of the mucosa, but higher on the nutrient than on the secretory side. This caused the loose membrane to balloon slightly into the secretory solution, so that the agitation produced by aeration effectively stirred the solution and prevented a tenacious layer of mucus from forming on the mucosal surface.

The outlet at the bottom of both chambers permitted convenient emptying, and, in the case of the secretory chamber, it provided a connection for a salt bridge for the measurement of pH. The bridge was filled with
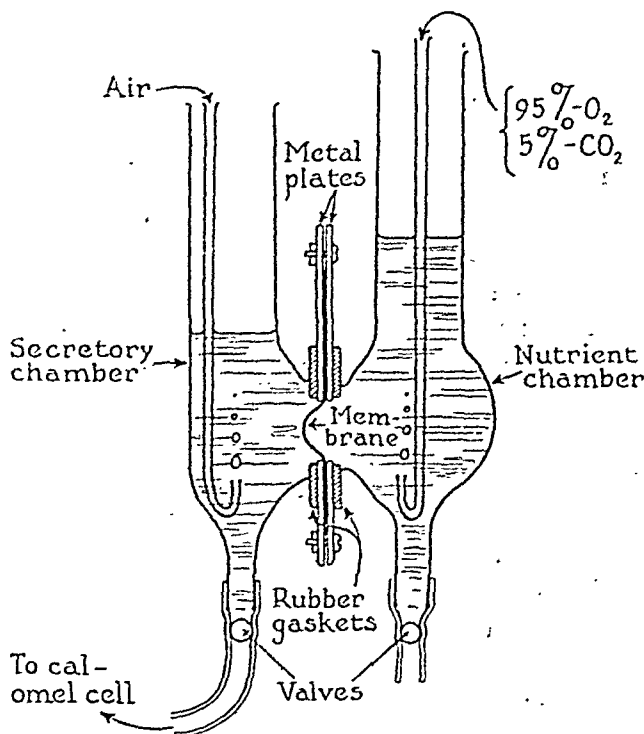
Fig. 1. Diagram of dual-chambered bath. When a pH determination is made, the aeration tube is removed and the glass electrode is inserted into the secretory chamber from the top.

the same solution as the secretory chamber (0.7 per cent NaCl). The junction between this solution and the reference cell was made by means of a KCl-agar cap as recommended by the Coleman Electric Company. The glass electrode was inserted into the secretory solution from the top, making it possible to record the pH at any time without withdrawing or contaminating the secretory solution. The pH readings were made with the Coleman pH Electrometer.

The nutrient solution had an ionic composition similar to that of frog's blood. Its high sodium bicarbonate content necessitated aeration with an oxygen-carbon dioxide mixture in order to maintain a pH of 7.4. The

solution contained, as millimols per liter, 71.75 of NaCl, 20.0 of NaHCO₃, 5.0 of KCl, 1.25 of CaCl₂, 1.20 of sodium phosphate buffer (pH 7.4), 0.80 of MgCl₂. Anhydrous glucose was added in a concentration of 50 mgm. per cent.

RESULTS. A large number of experiments have been made with various modifications of the apparatus and with various nutrient and secretory solutions. However, only results obtained with the method as described above, which yielded the most consistent and satisfactory results, will be given.

When the gastric mucosa of the frog was mounted as described, but not treated in any way, the pH of the secretory solution gradually fell over a period of hours and reached a plateau. In figure 2, the average of eight
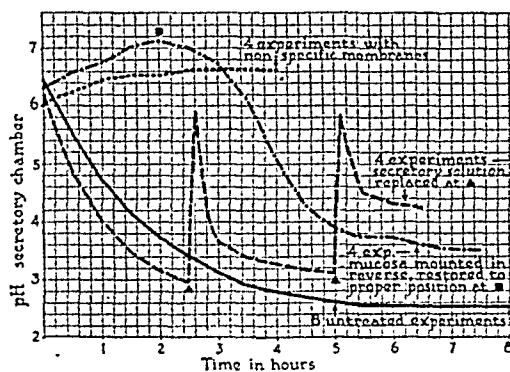
Fig. 2   Fig. 3

Fig. 2. The *in vitro* secretion of acid. The changes in pH of the secretory chamber during 4 different types of experiment are given.

Fig. 3. The *in vitro* secretion of acid by the "untreated" mucosa. The acidity is given in millimols/l. (clinical units) and the output of acid each half hour is given in thousandths of a millimol.
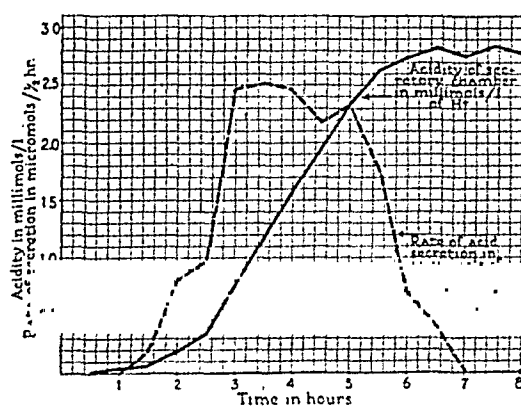
consecutive experiments of this type is presented in the form of a graph. The pH fell from an original value of 6.4 to a final value of 2.55. The highest acidities of the individual experiments ranged from pH 2.86 to 2.14. The highest acidity that has been observed is pH 1.6.

In figure 3, the development of acidity in the secretory solution has been plotted in terms of millimols per liter, instead of pH units. The rate of acid secretion, in terms of micro-mols (thousandth of a milli-mol) of H⁺ added to the bath each half-hour, is also shown. It is apparent that the mucosa does not begin to form appreciable amounts of acid until approximately two hours after being mounted, and that after 6 hours acid formation practically ceases.

The factors which are responsible for this "spontaneous" secretion have not yet been determined. The cessation of secretion, however, is not due

to an automatic suppression of further secretion by the presence of acid in the secretory solution. This is indicated by the fact that frequent renewal of the secretory solution does not alter the time at which secretion ceases (fig. 2).

Several types of experiment have yielded evidence which indicates that the *in vitro* production of acid represents a true secretory process. For example, non-specific membranes, such as the esophageal mucosa, the abdominal skin, or the abdominal musculo-peritoneal layer of the frog have failed without exception to form acid. In fact, the pH of the secretory solution has always increased (fig. 2). Of similar significance is our confirmation of Delrue (2) that gastric mucosa taken from winter frogs produces very little acid *in vitro*. It has been reported that the gastric glands of the intact frog do not secrete readily in winter (3).

Additional evidence that the *in vitro* production of acid is not the effect of a non-specific semi-permeable membrane was obtained in the following way. The gastric mucosa was mounted *in reverse* in the apparatus; the secretory surface was thus in contact with the nutrient solution and the muscularis surface in contact with the secretory solution. Under these conditions, in each of 14 experiments, the pH of the secretory solution rose. In fact, the solution became more alkaline than in the case of the non-specific membranes mentioned above, suggesting that alkali was being liberated from the muscularis surface. The fact that the pH of the buffered nutrient solution fell slightly supports this interpretation. After these membranes had been mounted in reverse for 2 hours, they were *restored* to the proper position. In ten cases the mucosa had apparently been killed and failed to secrete acid after this restoration; in four cases, however, the mucosa survived and produced significant amounts of acid (fig. 2).

DISCUSSION. The results reported here confirm and extend the observations of Delrue. This investigator recorded the pH of the secretory solution by means of a quinhydrone electrode, which necessitated the periodic removal of samples for the determination of pH. By the use of the glass electrode, which does not contaminate the solution tested, this complicating factor has been avoided. The nutrient solution used in this study conforms more closely to the ionic composition of frog's blood than the solution used by Delrue. The technique employed has yielded consistent and uniform results.

In the majority of experiments Delrue (1) obtained pH values ranging from 4.2 to 4.8, although occasionally lower values (pH 2.+) were obtained. This latter level he found to approximate the usual pH of the gastric contents of the frog. This was used as support for the belief that the *in vitro* acidity closely approximates the *in vivo* acidity of the frog's stomach. However, Friedman (4) has reported that pure gastric juice obtained

from the frog may have a free acidity of 72 millimols per liter, which represents a pH of 1.4. One could hardly expect to obtain this acidity *in vitro*, when the gastric secretion is diluted many times by the fluid of the secretory chamber.

In our experiments the acidity regularly fell considerably below the range of 4.2 to 4.8, for the average was 2.55. This difference is greater than appears superficially, due to the logarithmic nature of the pH unit. As can be seen from a comparison of the pH curve of figure 2 with the acidity curve of figure 3, a fall in pH from 6.0 to 4.0 represents an insignificant secretion of acid, whereas a fall from 3.5 to 2.5 represents the secretion of appreciable quantities of acid. In our experience, a pH below 3.0 has been followed by a gradual decrease in acidity after 8 to 10 hours. Delrue, on the other hand, reported that the plateau of acidity, presumably at higher pH levels, would persist for 24 hours or longer.

The production of sufficient acid to lower the pH of the secretory solution to an average value of 2.55, cannot be due to the formation of CO_2 by the dying mucosa. Irving (5) has shown that the pH of the gastric mucosa of the frog after death does not fall below pH 6.0. We have found that bubbling air through 0.7 per cent NaCl solution will not lower the pH below 6.0. If the formation of acid *in vitro* were a non-specific process, there is no reason why it should not have occurred when non-specific membranes were used, or when the mucosa was mounted in reverse.

CONCLUSIONS

1. The isolated gastric mucosa of the frog, when mounted *in vitro* in a suitable dual-chambered bath, formed sufficient acid to lower the pH of a saline solution to an average value of 2.55.

2. This formation of acid is believed to represent a genuine secretory activity on the part of the gastric glands for the following reasons: *a*, nongastric membranes fail to form acid; *b*, gastric mucosa taken from winter frogs forms little acid; *c*, the gastric mucosa exhibits a polarity such that acid is secreted only from the natural secretory surface, and alkali is liberated only from the reverse side.

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INFLUENCE OF AGE ON KETOSIS

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The fasting rat developes a ketosis. The extent of this ketosis may be determined by many factors. Age appeared to be a factor of possible importance. Black, Collip and Thomson (1) noted a definite increase in the degree of ketosis with age when it was measured by the ketonuria of rats on a butter diet. Heymann (2) has examined the influence of age on ketosis in man by a study of the ketonuria at various ages on a ketogenic diet. He found that ketosis was minimal in infancy, increases during the first four years of life to a maximum which lasts until about eight years of age and then decreases until it reaches the adult level at puberty. We have found the excretion of acetone bodies in the urine to be an uncertain and misleading indicator of the degree of ketosis which exists. Consequently we have examined the relation of ketosis to age in the albino rat using the acetone body level of the blood as a measure of the degree of ketosis. This figure represents the difference in the rate of production of acetone bodies by the liver and their rate of oxidation by the extrahepatic tissues.

EXPERIMENTAL. Male albino rats of known age were used. They had been receiving as a stock diet "Tioga dog pellets" (protein, 23.0; fat, 4.0; fiber, 4.0; ash, 12.5; moisture, 8.5; nitrogen-free extract, 48.0) and were fasted directly from this diet. Fasting was commenced at 9 o'clock in the evening but since rats generally sleep all day and eat at night they had already in one sense been some 12 hours without food when fasting was commenced. At twelve hour intervals groups were sacrificed for measurement of the acetone body level in the blood. The rats were anesthetized by an intraperitoneal injection of sodium pentobarbital and a blood specimen taken by severing the abdominal aorta. Total acetone bodies in the blood samples were determined by the method of Barnes and Wick (3) and liver glycogen by the method of Good, Kramer and Somogyi (4). Each group contained 3 rats except the 30 day old series in which there were six per group.

RESULTS. Averages of all pertinent data are presented in table 1. Following the onset of fasting at any age there is an increase in the level of acetone bodies in the blood. This increase tends to take place more

TABLE 1

The influence of age on the degree of fasting ketosis and the amount of glycogen in the liver

Group averages

AGE	HOURS OF FASTING								
	0	12	24	36	48	60	72	84	94
Blood acetone body concentration in milligrams per cent									
days									
30	0.3	15	25	29	23				
45		10	17	19	18	23	16	20	19
61	1.0	14	19	22	22	26	19	28	21
76	1.1	8	16	18	22	22	26	19	25
128	0.8	6	12	11	23	17	20	20	16
275	0.8	5	9	10	14	24	23	17	20
Liver glycogen concentration in grams per cent									
30	1.73	0.23	0.03	0.18	0.06				
45	2.90	0.17	0.04	0.13	0.36	0.51	0.57	0.43	0.30
61	2.00	0.11	0.04	0.11	0.25	0.21	0.06	0.33	0.11
76	2.80	0.34	0.03	0.13	0.05	0.31	0.10	0.75	0.15
128	3.30	0.79	0.09	0.13	0.05	0.36	0.16	0.53	0.43
275	1.80	0.66	0.06	0.19	0.18	0.13	0.05	0.23	0.19
Liver glycogen in milligrams per square decimeter body surface									
30	163.0	20.0	2.8	11.7	4.7				
45	160.0	9.2	2.0	6.7	15.8	24.0	23.7	18.1	12.1
61	109.0	5.8	1.8	5.0	12.2	9.7	2.3	14.0	4.3
76	176.0	18.9	1.5	6.8	2.4	15.7	4.3	39.0	6.2
128	196.0	26.0	3.3	5.7	2.4	14.9	6.3	22.0	11.3
275	110.0	34.0	2.7	8.7	8.7	6.4	1.9	8.1	8.1
Urine nitrogen in milligrams per square decimeter body surface per day									
30		27			31*				
45		31			26*		30*		
61		31			32		23*		24
76		31			29		25*		21*
128		26			23*		20*		23
275		35			25*		19*		18*
Liver weight in grams									
30	3.11	2.75	2.43	1.95	2.19				
45	5.16	4.83	4.00	4.00	3.53	3.66	3.33	3.10	3.16
61	6.46	5.44	4.90	5.00	5.13	4.66	4.20	4.30	4.00
76	7.97	7.16	6.17	6.35	5.66	5.89	5.00	5.94	4.76
128	8.65	7.05	5.55	5.59	5.71	5.68	5.38	5.52	5.15
275	11.21	9.66	8.74	8.43	8.74	8.55	7.44	7.51	7.60
Body weight in grams									
30	72	67	65	61	58				
45	121	112	106	99	97	92	96	87	90
61	169	158	156	148	147	142	136	137	132
76	199	195	189	183	182	174	173	167	166
128	241	232	226	221	216	215	207	206	198
275	348	341	336	333	327	322	321	316	316

rapidly, the younger the rat, but after the first 48 hours of fasting there is no significant difference in the degree of fasting ketosis at any age when it is measured by the blood acetone body level.

Urine collections were made over 24 hour periods primarily for nitrogen determinations. None of the specimens contained acetone bodies in measurable quantities but traces were present in all samples which in the nitrogen figures in table 1 are marked with an asterisk.

The changes in the amount of glycogen in the liver do not follow the variations in the acetone body concentration in the blood except for the tendency of the glycogen to fall more rapidly in young than in old animals. In all cases it reached a minimum after 24 hours of fasting and then tended to rise, in some cases increasing to significant amounts.

DISCUSSION. The decrease in the rate of onset of ketosis as the rats are older is undoubtedly due to the greater glycogen stores available for energy in the older rats. As these are exhausted and the organism is dependent on protein alone as a source of sugar the formation of acetone bodies reaches a common level. This suggests that after the first two days of fasting the relationship of the protein to fat which is utilized on further fasting must be the same at all ages. The urine nitrogen figures which are all of the same order of magnitude support this conclusion.

SUMMARY

The fasting ketosis of male albino rats as measured by the level of acetone bodies in the blood increases less rapidly the older the rat. After 48 hours of fasting it has reached a similar maximum for all ages.

The amount of glycogen in the liver tends to decrease somewhat less rapidly on fasting in old than in young rats. At all ages it reaches a minimum after 24 hours without food and then tends to increase for the next three days.

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THE EFFECTS OF ANGIOTONIN ON RENAL BLOOD FLOW AND GLOMERULAR FILTRATION

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Purified renin, the so-called renal pressor substance, does not cause vasoconstriction when perfused through the isolated rabbit's ear (Page, 1939). Addition of "renin-activator" (Kohlstaedt, Page and Helmer, 1940), a substance obtained from blood plasma, to solutions of renin permits full vasoconstrictor effect. Further study has shown that a crystalline pressor substance is obtained by the interaction of renin and renin-activator (Page and Helmer, 1940). This substance has been called angiotonin and is probably the effector substance of the action of renin.

Renin, when slowly infused intravenously into conscious, trained dogs, causes a prolonged decrease of renal blood flow which is in great part the result of efferent glomerular vasoconstriction (Corcoran and Page, 1939a, 1940). The renal action of angiotonin was therefore examined by similar methods. It was expected that the action of angiotonin would in some respects differ from that of renin, since its liberation into the blood would probably be slower and more uniform when presented by renin infusion than when given as such. A general similarity of renal effects would support the view that renin acts by liberation of angiotonin.

METHODS. The methods used were those previously described in detail (Corcoran and Page, 1939). Renal blood flow (C/Ep 100), phenol red and inulin clearances $\left(C = \dfrac{UV}{P} \right)$, and renal extraction percentages $\left(\dfrac{A-V}{A} \ 100 \right)$ were determined in trained, uninephrectomized female dogs whose remaining kidney had been subcutaneously explanted by the method of Page and Corcoran (1940). Plasma and urine phenol red and inulin concentrations were found by the methods of Corcoran and Page (1939a, b).

Each experiment consisted of two or more control periods, during which phenol red and inulin in saline solution were infused at the rate of 1 cc. per minute, their renal clearances and extractions determined, and renal blood and plasma flows found by calculation. Angiotonin, prepared by Dr. O. M. Helmer, was then added to the infusing fluid, and the observa-

tions continued. Blood pressures were recorded once in each period by femoral arterial puncture.

RESULTS. The results of two experiments are presented graphically (fig. 1). The protocols of these and of 3 other experiments are tabulated at the end of this paper. Several experiments done with weak solutions of angiotonin shortly after its existence was recognized resulted in only transient increases of arterial pressure and fleeting renal responses. These are not included in this report. Experiments done with larger doses of angiotonin, in which sustained increases of arterial pressure were observed,
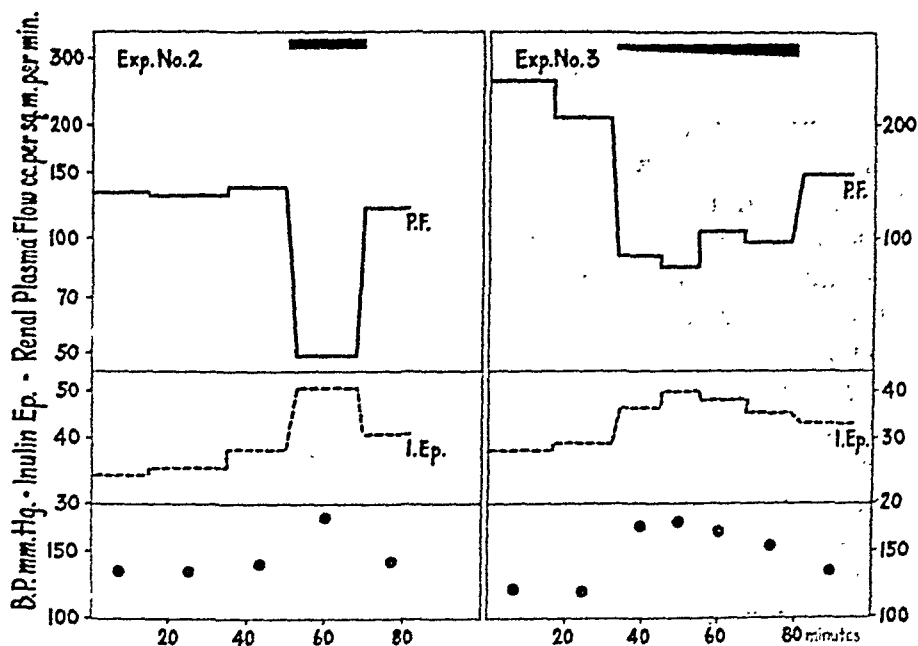
Fig. 1. Effect of infusion of angiotonin on renal plasma flow, inulin extraction percentage and femoral arterial blood pressure. *P. F.*, renal plasma flow; *I. Ep.*, inulin extraction percentage; *B. P.*, femoral arterial pressure millimeters of mercury. Period of angiotonin infusion indicated by shaded area.

always resulted in decreased renal blood flow and increased renal extraction of inulin.

The infusion of angiotonin at a steady pace reduced renal blood flow, increased arterial pressure and renal extraction of insulin. Interruption of the angiotonin infusion promptly results in a return of blood pressure, renal blood flow, and renal extraction towards normal.

It will be noted that renal extraction of phenol red is relatively high during angiotonin infusion, even at plasma levels at which the load of phenol red brought in the plasma to the renal secretory cells is so large that the proportion removed from blood ordinarily decreases (above 1.5 mgm. per 100 cc.). We have previously observed increased renal extraction of phenol red during renal ischemia at plasma levels below 1.5 mgm.

TABLE 1

Effects of angiotonin infusion on renal plasma flow, phenol red and inulin clearances and extraction percentages

Time in minutes from each emptying of the bladder. Angiotonin—relative amounts infused indicated by plus sign. P.R.C., plasma phenol red clearance; P.R.Ep., phenol red plasma extraction percentage; P.P.R., arterial plasma phenol red in milligrams per 100 cc.; I.C., plasma inulin clearance; I.Ep., plasma inulin extraction percentage; P.F., renal plasma flow; mean of values calculated from phenol red and inulin clearances and extractions; B.P., mean femoral arterial pressure in millimeters of mercury. Values for renal clearance and plasma flow expressed as cubic centimeters per square meter body surface per minute. Decimal fractions used in the calculation of renal plasma flow are not included in the table.

EXPERIMENT NUMBER	TIME	ANGIOTONIN	P.R.C.	P.R.EP.	P.P.R.	I.C.	I.EP.	P.F.	B.P.
1	0–18	0	59	50	0.872	35	26	126	115
	18–39	0	50	49	0.828	32	33	98	108
	42–64	+	20	78	2.46	19	44	34	158
	64–79	+	53	54	3.12	34	44	86	148
2	0–15	0	92	64	0.530	42	34	133	134
	15–35	0	82	59	0.623	43	35	130	133
	35–50	0	97	65	0.865	48	38	136	140
	53–68	++	32	77	2.79	29	51	49	187
	70–82	0	71	59	4.61	45	41	115	141
3	0–17	0	131	49	0.670	72	28	262	121
	17–32	0	94	50	0.820	67	29	207	118
	34–45	+	50	63	1.27	45	36	91	167
	45–55	++	40	56	1.95	38	40	85	171
	55–67	++	62	52	2.14	36	38	106	160
	67–79	+++	55	63	2.81	49	35	99	155
	82–95	0	74	51	2.23	52	33	150	134
4	0–19	0	87	45	0.860	62	34	186	121
	19–35	0	89	43	0.913	64	33	200	118
	35–52	0	75	43	0.958	53	32	172	120
	55–67	+	36	60	2.01	39	42	76	171
	67–79	+	47	54	2.92	59	44	110	160
	79–91	+	51	57	3.84	53	44	104	155
5	0–13	0	113	52	0.692	55	28	204	124
	13–27	0	135	59	0.650	67	28	235	121
	27–45	0	99	47	0.461	56	27	212	132
	48–59	+	61	54	0.753	34	46	100	142
	59–75	+	70	55	0.822	26	36	99	139

per 100 cc. and have attributed this effect to a relative delay of the blood stream in the peritubular capillaries. The increased renal extraction of

phenol red observed during the renal ischemia produced by angiotonin has probably a similar mechanism.

As with renin (Landis, Montgomery and Sparkman, 1938), infusion of angiotonin causes no consistent change in skin temperature, which was measured in a few experiments. Large doses caused salivation, and occasionally retching, which were also observed with large doses of renin.

DISCUSSION. Infusion of angiotonin causes renal ischemia which, at least at the start of the infusion, may be profound, although it tends to lessen as the infusion is continued. The degree of ischemia is relatively greater than that observed during infusion of renin in doses which produced the same percentile increase of arterial pressure. The action of angiotonin seems also more fleeting than that of renin, since both renal blood flow and arterial pressure tend promptly to return towards normal after interruption of the infusion, although, as with renin (Merrill, Williams and Harrison, 1938), the return of renal blood flow lags behind the return of arterial pressure. These observations are consistent with the action of a substance which, when presented during renin infusion, is slowly liberated within the body, and, when presented as such, acts more intensely and ceases acting as soon as it is withdrawn because there is no circulating source of the material as there is when renin has been given.

The increased renal extraction of inulin observed during angiotonin infusion is similar to that observed during infusion of renin. Removal of inulin from blood in the kidney occurs by glomerular filtration from the plasma water, and variations in its removal must result from variations of intraglomerular pressure. Since arterial hypertension, presumably due to increased cardiac output, has relatively little effect on intraglomerular pressure as measured by inulin extraction (Corcoran and Page, 1939a), the changes observed during angiotonin infusion occurred as the result of circulatory adjustments within the kidney, either by dilatation of the afferent or constriction of the efferent arterioles. The co-existence of renal ischemia with increased intraglomerular pressure strongly suggests that the increased filtration pressure, and, in some measure at least, the restriction of renal blood flow occurred as the result of constriction of glomerular efferent arterioles. Since in some experiments as much as 45 per cent of the arterial plasma inulin (and water) was removed in the kidney by filtration, the increase in intraglomerular pressure must have been very great.

The renal action of angiotonin is, then, similar to that of renin and differs only in those characteristics by which one would expect an effector substance to differ from a material from which it is slowly liberated. The renal effects of angiotonin and renin are similar to the mechanisms which obtain in arterial hypertension in human beings (Smith, Goldring, Chasis and Ranges, 1938; Goldring, Chasis, Ranges and Smith, 1938), namely, decreased renal blood flow and increased filtration fraction (renal extrac-

tion of inulin), both, apparently, due to efferent arteriolar constriction. It is of interest to note from the observations of Smith and colleagues and from our own unpublished data that renal extraction of phenol red is also increased in these cases, probably by a mechanism similar to that operating during the infusion of angiotonin and renin.

SUMMARY

Infusion of angiotonin into conscious, trained dogs with explanted kidneys, results in increased arterial pressure, decreased renal blood flow, and increased extraction of inulin from blood by the kidney. These effects are similar to those observed during infusion of renin, although with angiotonin the effects were initially more profound, and, after interruption of the infusion, more fleeting. The increased extraction of inulin and restriction of renal blood flow which occur during angiotonin infusion are, in great part, the result of efferent glomerular vasoconstriction. The effects of angiotonin on the kidney support the view that it is the effector substance of renin action.

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OSMOTIC RELATION BETWEEN AQUEOUS HUMOR AND BLOOD PLASMA[1]

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Gilman and Yudkin (1933), using the Hill (1930) method of measuring vapor pressure, found the aqueous humor of dogs to be isosmotic to the blood. Benham, Davson, and Duke-Elder (1937), using Baldes' (1934) modification of the thermoelectric method, found the aqueous humor of cats under ether anesthesia to be hyposmotic to the blood serum. However, in a more recent study, Benham, Duke-Elder and Hodgson (1938) found that under local anesthesia the aqueous humor was hyperosmotic to blood serum and concluded that aqueous humor was formed by secretion rather than by filtration. They explained their earlier results as being due to the fact that ether anesthesia results in an increase in the osmotic activity of the blood.

Investigators who have studied the distribution of ions between blood and aqueous humor also disagree as to the mechanism of aqueous humor formation. Hodgson (1938) finds the ratio of chloride in the aqueous humor to that in the blood serum to be greater than that expected on the basis of ultrafiltration. Davson (1939) believes that the distribution of sodium and chloride between serum and aqueous humor does not justify the assumption of a secretory mechanism for the formation of aqueous humor. Walker (1933) found the concentration of urea in the aqueous humor of rabbits, dogs and man to be on the average 30 per cent less than in the plasma. Similar differences were observed in the concentration of uric acid in the aqueous humor and plasma of fowls and man and in the concentrations of inorganic phosphates and reducing substances in the aqueous humor and plasma of frogs. From these results Walker concluded that aqueous humor is not formed by simple filtration or dialysis.

In attempting to throw further light on the mode of formation of aqueous humor, we have repeated some of the work of Benham et al. (1938) and, in addition, we have studied the osmotic relationship between aqueous humor and blood plasma following the injection of a poison, $HgCl_2$, into the anterior chamber of the eye.

EXPERIMENTAL PROCEDURE. Determinations of osmotic activity were made by the thermoelectric method as modified by Baldes (1934) and Baldes and Johnson (1939). All determinations were made in the presence of 5 per cent CO_2 in oxygen and the results are expressed in terms of the concentration of an isosmotic solution of NaCl. Blood samples were drawn in an oiled syringe from the femoral artery. The blood was trans-

TABLE 1

Comparison of osmotic activity of aqueous humor and blood plasma with animals under Nembutal anesthesia

ANIMAL	TIME	OSMOTIC ACTIVITY IN mM NaCl PER KILO H_2O		
		Plasma	Aqueous humor	Difference
	minutes			
Rabbit 1...............		155.6	158.5	+2.9
Rabbit 2...............		164.7	165.9	+1.2
Rabbit 3...............	0	147.2	148.6	+1.4
	32	147.6	150.0	+2.4
Rabbit 4...............	0	164.0	165.8	+1.8
	32	163.7	165.3	+1.6
Average, 4 rabbits				+1.9
Dog 1...................	0	160.6	R 164.0	+3.4
	27	160.9	L 164.9	+4.0
	72	161.0	R 165.3	+4.3
Dog 2...................	0	160.6	R 163.3	+2.7
	54	159.8	L 163.7	+3.9
Dog 3...................	0	160.0	R 164.0	+4.0
	37	159.3	R 160.9	+1.6
	79	159.0	R 162.3	+3.3
Dog 11.................	0	161.3	L 164.7	+3.4
	29	163.2	L 166.1	+2.9
	93	164.4	L 166.7	+2.3
Average, 4 dogs				+3.3

ferred to a cooled test tube containing oil and a small amount of dried heparin (Connaught), the tube was stoppered and the plasma separated immediately by centrifugation. Samples of aqueous humor were removed with a tuberculin syringe by lateral insertion into the anterior chamber.

In adding $HgCl_2$ to the aqueous humor in the anterior chamber, a measured volume (0.01–0.05 cc. as indicated in the table of results) of a 0.1 M solution of $HgCl_2$ was placed on the end of the plunger of a 2 cc.

syringe and evaporated to dryness. A portion of the aqueous humor
(0.3–0.8 cc.) was then drawn into the syringe, the HgCl₂ dissolved by ro-
tating the syringe and the solution reinjected into the anterior chamber.
Nembutal was used as the general anesthetic and procain (3 per cent solu-
tion) as the local anesthetic.

RESULTS AND COMMENT. The results obtained by comparing the osmotic
activity of aqueous humor and blood plasma of rabbits and dogs under
Nembutal are given in table 1. In every instance the osmotic activity of
the aqueous humor was greater than that of the blood plasma. Since
changes in the osmotic activity of aqueous humor would necessarily lag
behind any changes occurring in the blood plasma, several series of sam-
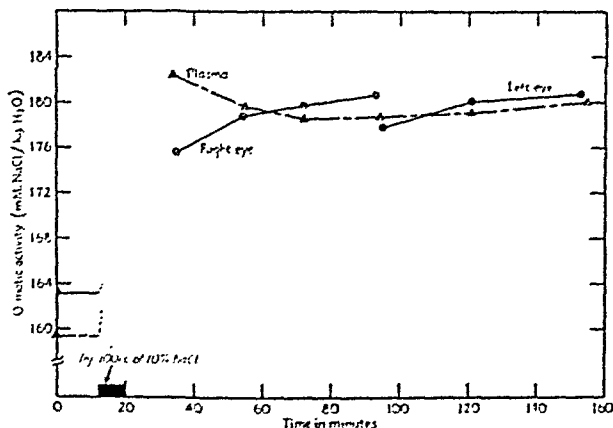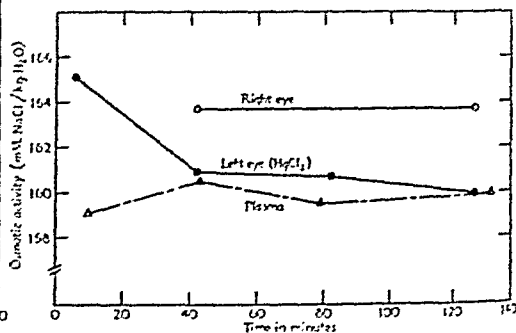
Fig. 1   Fig. 2

Fig. 1. Changes in osmotic activity of aqueous humor and blood plasma of dog
following intravenous injection of 5.0 cc. of 10 per cent NaCl per kilo of body weight.
(Kidneys ligated.)

Fig. 2. Osmotic relation between aqueous humor and blood plasma of dog. Seven-
tenths cubic centimeter of aqueous humor removed from left eye and reinjected with
0.003 milli-mol HgCl₂ posterior to iris.

ples were collected over a period of time in order to be sure that the osmotic
activity of the plasma was relatively constant. Since only 0.05–0.10 cc.
of aqueous humor was removed for each determination, several samples
could be taken from the same eye.

Changes in the osmotic activity of aqueous humor and blood plasma
following the intravenous injection of hypertonic NaCl are shown in
figure 1. In this experiment the kidneys were ligated in order that the
osmotic activity of the plasma would remain constant at the elevated level.
It should be noted that if the volume of aqueous humor in the eye is not
reduced by the removal of samples, more than 80 minutes is required for
the aqueous humor to become again hyperosmotic to the blood plasma.

It seemed possible that excitement of the animal accompanying the

withdrawal of aqueous humor from untrained dogs under local anesthesia might result in an increase in the osmotic activity of the plasma above that of the aqueous humor. With this in mind an attempt was made to duplicate the results of Gilman and Yudkin (1933) who obtained their samples under local anesthesia. The results of these experiments are given in table 2. It was found that under local anesthesia the difference in osmotic activity between aqueous humor and plasma is even greater than under Nembutal anesthesia. With dogs 7 and 8 two samples of blood were taken, one before and the other immediately after the removal of the aqueous humor. With dogs 9 and 10, the blood samples were taken immediately following the removal of the aqueous humor and in these experiments the dogs became excited and struggled appreciably while the

TABLE 2

Comparison of osmotic activity of aqueous humor and blood plasma of dogs under local anesthesia (Procaine)

DOG NO.	OSMOTIC ACTIVITY IN mM NaCl PER KILO H_2O		
	Blood plasma	Aqueous humor	Difference
7	158.0 157.3	162.8	+5.1
8	153.3 152.9	159.8	+6.7
9	154.7	161.0	+6.3
10	152.8	156.3	+3.5
Average..................................			+5.4

samples were being taken. Dog 10 was recovering from distemper at the time of the experiment.

Although the osmotic activity of the aqueous humor is found to be consistently higher than that of the blood plasma, the question arises as to whether this difference, as measured, actually exists in vivo. One may not be justified in assuming that the blood removed from the femoral artery has the same osmotic activity as the blood flowing through the optic artery. In order to show more conclusively that the difference in osmotic activity between aqueous humor and blood plasma is real and is indicative of an active secretory process, we studied the effect of injecting $HgCl_2$ into the anterior chamber upon this difference in osmotic activity. As shown by the data given in table 3 and the graph in figure 2, the effect of $HgCl_2$ is to reduce or abolish the difference in osmotic activity between the plasma and aqueous humor. In most of the experiments the aqueous humor and

$HgCl_2$ were injected into the anterior chamber posterior to the iris since the injection of $HgCl_2$ anterior to the iris does not appear to be as effective in reducing the difference in osmotic activity. This is shown by the results obtained with dogs 3 and 6, and is probably due to the circulation of aqueous humor from the ciliary bodies posterior to the iris into the chamber anterior to the iris (Best and Taylor, 1939).

The concentration of $HgCl_2$ in the aqueous humor necessary to abolish the difference in osmotic activity is of the same order of magnitude although slightly higher than that found by Ingraham and Visscher (1936) to poison the absorption of chloride from the ileum of dogs.

TABLE 3

The effect of injection of $HgCl_2$ into the anterior chamber of the eye on the difference in osmotic activity between aqueous humor and blood plasma

DOG	TIME	OSMOTIC ACTIVITY IN mM NaCl PER KGM. WATER				
		Plasma	Aqueous humor		Difference	
			Left eye	Right eye	Left eye	Right eye
	*minutes**					
3	35	160.0	161.9†	164.0	+1.9	+4.0
	73	159.3	160.6	160.9	+1.3	+1.6
	114	159.0	160.9	162.3	+1.9	+3.3
4	135	158.9	158.9‡	163.7	0.0	+4.8
5	90	159.1	160.5§	163.9	+1.4	+4.8
6	160	160.4	160.8¶	161.4‖	+0.4	+1.0
12	100	157.1	157.2**	160.6	+0.1	+3.5
	142	158.1	156.6	160.8	−1.5	+2.7

* Time following injection of $HgCl_2$ into anterior chamber of left eye.
† 0.8 cc. of aqueous humor removed and reinjected with 0.0025 milli-mols $HgCl_2$ anterior to iris.
‡ 0.7 cc. of ditto with 0.003 milli-mol $HgCl_2$ posterior to iris.
§ 0.5 cc. of ditto with 0.001 milli-mol $HgCl_2$ posterior to iris.
¶ 0.3 cc. of ditto with 0.001 milli-mol $HgCl_2$ posterior to iris.
‖ 0.5 cc. of ditto with 0.005 milli-mol $HgCl_2$ anterior to iris.
** 0.5 cc. of ditto with 0.002 milli-mol $HgCl_2$ posterior to iris.

These results indicate that aqueous humor is not an ultrafiltrate of blood plasma. In the formation and circulation of aqueous humor there appears to be a net transport of a hyperosmotic solution from the blood plasma into the anterior chamber of the eye. This mechanism may be similar to that involved in the absorption of chloride from the intestine where differences in total osmotic activity also occur. It has been shown that during the absorption of chloride from the ileum against a concentration gradient, the osmotic activity of the intestinal fluid decreases below that of the blood plasma (Roepke and Visscher, 1939). In that case there is a net movement of hyperosmotic solution into the blood.

The authors are indebted to Prof. M. B. Visscher for his helpful suggestions and criticisms.

SUMMARY

The osmotic activity of aqueous humor was found normally to be higher than that of the blood plasma. The re-injection of aqueous humor containing $HgCl_2$ into the anterior chamber posterior to the iris was found to abolish the difference in osmotic activity between aqueous humor and blood plasma.

The results support the view that active secretory processes are involved in the formation of aqueous humor.

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ANALYSIS OF THE VASOPRESSOR AND OTHER "NICOTINIC" ACTIONS OF ACETYLCHOLINE

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Hunt (1) observed that large doses of acetylcholine caused a rise rather than a fall in blood pressure in animals in which the vasodilator action of this drug was opposed by atropine. Numerous other early authors (2, 3, 4, 5, 6 and 7) described the vasopressor effect of choline and some of its derivatives in atropinized animals. Dale (7) distinguished between the "muscarine-like" (vasodilator and other parasympathomimetic actions) and the "nicotine-like" effects of acetylcholine. He assumed that the latter effects were due to a ganglionic stimulant action of acetylcholine and showed that doses of nicotine sufficient to paralyze autonomic ganglia abolished the vasopressor effects of acetylcholine.

More recently, Simonart (8), and Feldberg and Minz (9) confirmed the observations of Dale and others, but believed that at least part of the acetylcholine rise was due to mobilization of epinephrine from the suprarenal glands.

The assumption of Hunt and Dale that the vasopressor action of acetylcholine is due to ganglionic stimulation received confirmation by the work of Feldberg and Gaddum (10), and by Koppanyi, Dille and Linegar (11). The latter have shown that the depression or paralysis of the superior cervical ganglion by nicotine may be overcome by large doses of acetylcholine or physostigmine. The present work was undertaken to extend these observations to the vasomotor sympathetic ganglia and to analyze the vasopressor and other "nicotine-like" actions of acetylcholine.

EXPERIMENTAL. Dogs and cats were used throughout the experiments. In most cases sodium pentobarbital (30–35 mgm.[1]) administered intravenously was used as an anesthetic; in others sodium amytal (50 mgm. by vein) or urethane (from 0.75–1.00 gram intraperitoneally) was used. The drugs used were administered intravenously in all cases with the exception of cocaine hydrochloride, which was also given intramuscularly.

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[1] All doses are expressed in terms of milligrams per kilogram of body weight, unless otherwise stated. To avoid repetition the words "per kilogram of body weight" are omitted.

In all animals the blood pressure was recorded from the common carotid artery and the respiration by connecting a tracheal cannula with a Marey tambour. Duodenal motility was recorded with a water manometer connected to a balloon inserted into the upper duodenum through the stomach.

In several experiments it was necessary to inject fixed quantities of arterial blood of one animal into a vein of another. This was done by inserting a three-way glass cannula into the common carotid artery of the donor and into the femoral vein of the recipient (dogs or cats) with each cannula connected by small rubber tubing to a metal three-way T-valve to which a 30 cc. syringe was attached. A clamp was placed on the cannulated carotid artery between injections. By releasing the clamp on the artery and opening the valve of the metal T-tube on the arterial side, the syringe was allowed to fill to the desired volume, the arterial valve was closed, the valve connected with the femoral vein opened, and the blood injected into the recipient at a constant rate. Careful cleaning of the cannulae and prompt saline infusion following the transfusion made it unnecessary to use an anticoagulant.

A. *The action of physostigmine on the acetylcholine pressor effect.* It is a well known fact that doses of acetylcholine from 1 to 5 mgm. produce a marked vasopressor effect in atropinized animals. In these animals (2 to 5 mgm. of atropine sulfate) under barbiturate or urethane anesthesia we have found that 0.5 mgm. of acetylcholine chloride usually produced pressor effects, but that 0.15 mgm. or less did not produce pressor responses except for small rises in an occasional animal. It was observed, however, that when an atropinized animal was given 0.5 to 1.0 mgm. of physostigmine salicylate or prostigmin, acetylcholine in doses of 0.025 to 0.1 mgm. produced sharp epinephrine-like rises in blood pressure of 30 to 120 mm. of mercury (see fig. 1). Occasionally, with optimum amounts of physostigmine, definite pressor effects were seen following the injection of 0.001 to 0.005 mgm. of acetylcholine.

In animals in which the amounts of atropine (5.0 mgm.) and acetylcholine (0.05 mgm.) were being kept constant, physostigmine was administered in gradually increasing amounts from 0.005 mgm. upward. The results showed that in the average animal from 0.03 to 0.04 mgm. of physostigmine was necessary to obtain a definite elevation of blood pressure from 0.05 mgm. of acetylcholine. Doses of 0.02 mgm. of physostigmine or less were ineffective, while with up to 0.5 mgm. of physostigmine the magnitude of the acetylcholine pressor effect increased. With 0.5 to 15 mgm. of physostigmine the acetylcholine vasopressor effects were about optimal, while beyond that point they began to decline. Artificial respiration was often necessary after the animal had received 4 to 6 mgm. of physostigmine or 1 to 2 mgm. of prostigmin.

The doses of atropine used in all experiments varied from 0.5 to 60 mgm. In general large doses of atropine did not appear to interfere either with the vasopressor action of acetylcholine or the action of physostigmine, unless it produced toxic effects with marked fall in blood pressure.

In addition to acetylcholine two other choline derivatives with "nicotine-like" action, viz., muscarine (nitrous acid ester of choline) and doryl (carbamyl choline chloride), were given in doses of 0.05 to 1 mgm. to atropinized animals. The minimum dose of muscarine producing a rise in blood pressure in atropinized dogs lies between 0.1 to 0.5 mgm. and of doryl 0.05 to 0.2 mgm. One-half to 1.0 mgm. of muscarine produced
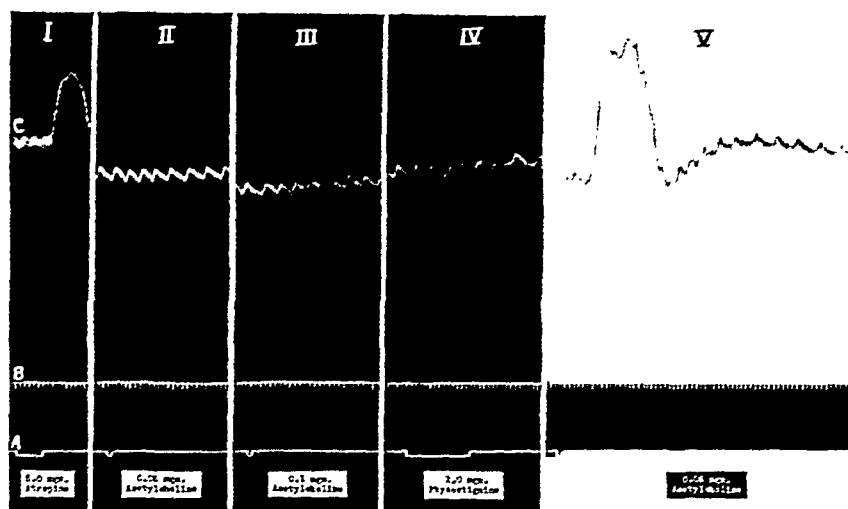
Fig. 1. Dog, 9.6 kgm., 30 mgm. of sodium pentobarbital per kilogram by vein. A, line indicating injection of drugs. B, time, 2 seconds; base line representing zero mm. of Hg pressure. C, blood pressure tracing from the common carotid artery.

I, 5.0 mgm. of atropine sulfate per kilogram, vein. II, two minutes later; 0.05 mgm. of acetylcholine chloride per kilogram, vein. III, one minute later; 0.1 mgm. of acetylcholine chloride per kilogram, vein. IV, one minute later; 1.0 mgm. of physostigmine salicylate per kilogram, vein. V, five minutes later. 0.05 mgm. of acetylcholine chloride per kilogram, vein.

pressor effects ranging from 30 to 70 mm. of mercury, and 0.5 to 1 mgm. of doryl rises in blood pressure of 40 to 120 mm. Physostigmine in doses of 0.1 to 5 mgm. did not increase the magnitude of the pressor responses of either muscarine or doryl.

B. *Phenomena accompanying the pressor effect of acetylcholine.* In addition to the elevation of blood pressure, the animals treated with atropine and physostigmine show central stimulation, increase in respiration (16), stimulation of the ocular sympathetics, relaxation of the gut and cardiac acceleration following the injection of 0.05 mgm. of acetylcholine.

In a number of experiments in which the duodenal motility was recorded,

acetylcholine in the presence of small amounts of atropine and relatively large amounts of physostigmine produced a distinct phase of relaxation of the intestine (see fig. 2). In those cases in which the pressor effect of acetylcholine was preceded by a marked fall in blood pressure, this intestinal relaxation occurred before the blood pressure elevation. In control experiments when pressor responses were produced by rapid injection of 7 per cent acacia solution there was no effect on intestinal motility and when blood pressure falls were produced by injections of acetyl-beta-methylcholine there was only an increase in duodenal activity (see fig. 2).
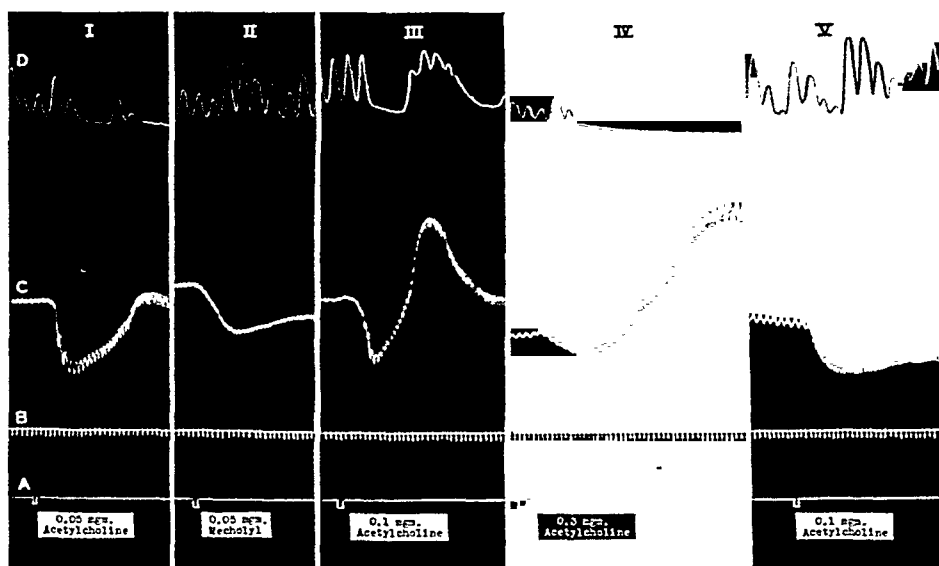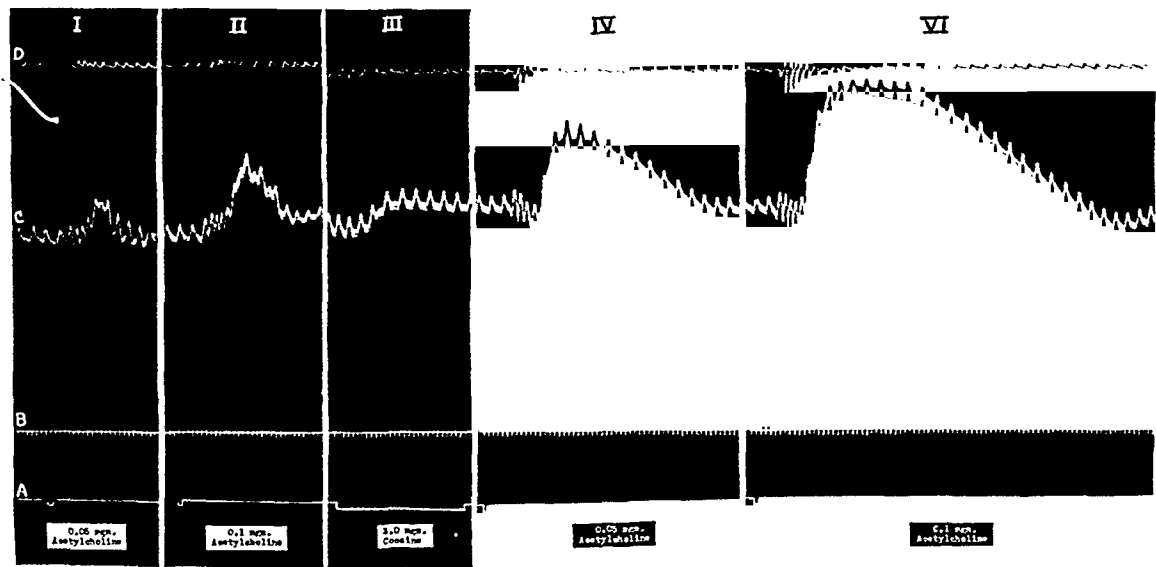

Fig. 2. Dog, 8.6 kgm., 35 mgm. of sodium pentobarbital, 0.1 mgm. of atropine sulfate and 0.5 mgm. of prostigmin per kilogram by vein.

A, line indicating injection of drugs. B, time, 2 seconds; base line representing zero mm. of Hg pressure. C, blood pressure tracing from the common carotid artery. D, tracing of duodenal motility.

I, 0.05 mgm. of acetylcholine chloride. II, 0.05 mgm. of acetyl-beta-methylcholine chloride. III, 0.1 mgm. of acetylcholine chloride. IV, 0.3 mgm. of acetylcholine chloride. V, following 3.0 mgm. of nicotine salicylate; 0.1 mgm. of acetylcholine chloride.

Nicotine in doses of 1 to 10 mgm. abolished the pressor effect of acetylcholine and the accompanying intestinal relaxation.

C. *The duration of action of physostigmine in atropinized animals.* In two dogs receiving sodium pentobarbital, the duration of action of 1.0 mgm. of physostigmine upon the vasopressor effect of 0.05 mgm. of acetylcholine following 3.0 mgm. of atropine was found to be about six hours. The acetylcholine effects did not diminish in height until about the end of the physostigmine action, but the latent period between injection of acetylcholine and the beginning of the blood pressure rise was lengthened,

and the rise in blood pressure was more gradual though of the same height. At the end of physostigmine action acetylcholine produced a small rise, no rise, or even a fall in blood pressure. Additional physostigmine restored the pressor effects without the necessity of another atropine injection.

Similarly the vasopressor effect of acetylcholine was preserved after clamping the abdominal aorta at the level of the diaphragm in one cat and two dogs and remained undiminished for two hours or more until the animals began to fail.

D. *The action of cocaine on the vasopressor response to acetylcholine.* Cocaine hydrochloride, if administered to animals in doses of 3.0 mgm. intravenously or 5 to 10 mgm. intramuscularly, produced a gradual rise in blood pressure with occasional cardiac irregularities. If the above doses of cocaine were administered to animals already under the full effects of atropine, subsequent injections of 0.025 to 0.15 mgm. of acetylcholine did not produce pressor effects. In several cases, it was observed that doses of acetylcholine producing no hemodynamic effects before cocaine produced depression of blood pressure following cocaine. This latter effect may be attributed to the cardiac and probably vascular toxic effects of cocaine.

In all experiments when vasopressor effects from 0.025 to 0.15 mgm. of acetylcholine were established by previous administration of atropine and physostigmine or prostigmin, cocaine enhanced and prolonged these vasopressor responses (see fig. 3). Occasionally these potentiated pressor effects were preceded by falls of blood pressure. Cocaine in a few experiments potentiated the pressor effects of both muscarine and doryl.

E. *Locus and mode of the vasopressor action of acetylcholine.* Acetylcholine pressor effects are similar in appearance and duration to those obtained with epinephrine or nicotine. This epinephrine-like action may be due to central effects, carotid sinus reflexes, mobilization of epinephrine from the suprarenal glands or of other pressor substances, e.g., from the liver, or to stimulation of any part of the sympathetic neurone by acetylcholine directly, or to a combination of these factors. To determine the part these factors may play in the elicitation of the acetylcholine pressor effect, the following group of experiments was performed.

1. *Carotid sinus and central effects.* In seven cats and nine dogs both carotid sinuses were removed, both superior cervical ganglia resected and the vagi cut above the ganglia nodosa. In these animals with blood pressures of about 150 mm. of mercury and treated previously with 5 mgm. of atropine and 1 mgm. of physostigmine, 0.1 mgm. of acetylcholine produced blood pressure elevations of 60 mm. of mercury or more.

In two cats the entire cerebrum, cerebellum, medulla and spinal cord were destroyed in an acute experiment, the animals being kept alive by artificial respiration and continuous intravenous infusion of 7 per cent gum

acacia solution in saline.   In these two animals previously treated with 5 mgm. of atropine and 2 mgm. of physostigmine, 0.05 to 0.2 mgm. of acetylcholine produced rises up to 40 mm. of mercury which persisted after clamping the abdominal aorta.   In these animals the blood pressure elevations were not accompanied by respiratory stimulation and movements of the animals.

2. *Removal of the adrenals or liver and evisceration by clamping the abdominal aorta.*   The following experiments were performed in nembutalized animals treated with physostigmine (1 mgm.) and atropine (5 mgm.)

Fig. 3. Dog, 11.75 kgm., 30 mgm. of sodium pentobarbital, 5.0 mgm. of atropine sulfate and 1.0 mgm. of physostigmine salicylate per kilogram by vein.

A, line indicating injection of drugs.   B, time, 2 seconds; base line representing zero mm. Hg pressure.   C, blood pressure tracing from the common carotid artery. D, respiratory tracing from a tracheal cannula.

I, 0.05 mgm. of acetylcholine chloride per kilogram, vein.   II, one minute later; 0.1 mgm. of acetylcholine chloride per kilogram, vein.   III, two minutes later; 3.0 mgm. of cocaine hydrochloride per kilogram, vein.   IV, two minutes later; 0.05 mgm. of acetylcholine chloride per kilogram, vein.   V, one minute later; 0.1 mgm. of acetylcholine chloride per kilogram, vein.

and in most cases under artificial respiration, particularly after clamping of the aorta.

a. In ten dogs and five cats clamping the abdominal aorta at the level of the diaphragm did not prevent pressor effects from 0.05 mgm. of acetylcholine injected into the external jugular vein.   The rises were sometimes smaller, but often as great or greater than those obtained previous to clamping of the aorta in spite of the higher blood pressure level.   In both cats and dogs pressor effects from acetylcholine after clamping the abdominal aorta could be elicited as long as the animals lived.

b. In three dogs the entire liver was removed in acute experiments and the abdominal aorta clamped at the level of the diaphragm. In these animals 0.05 to 0.2 mgm. of acetylcholine produced blood pressure rises from 10 to 60 mm. of mercury.

c. In three cats and two dogs the skin of the anterior part of the animal was removed. Acetylcholine (0.05 mgm.) was then injected into the animals previously treated by atropine and physostigmine and the usual pressor effects obtained. Following clamping of the abdominal aorta, the same dose of acetylcholine usually produced a steep fall in blood pressure, whereas 0.1 cc. (total dose) of a 1:10,000 solution of epinephrine or 0.2 mgm. of nicotine produced rises of blood pressure. In one dog immediately after clamping the aorta acetylcholine produced pressor effects twice and further injections produced falls.

d. In fifteen animals both adrenals were removed and the blood pressure was kept above the shock level either by continuous intravenous infusion of 7 per cent acacia solution in saline or by clamping the abdominal aorta just below the diaphragm. In all these animals 0.05 to 0.1 mgm. of acetylcholine produced blood pressure elevations from 10 to 60 mm. of mercury. In other animals in which the adrenals were removed or the adrenal veins clamped and the resulting blood pressure fall was not compensated by either of the two above measures, acetylcholine produced a small or no rise in blood pressure. In one animal both adrenals were removed and the blood pressure rose to 90 mm. of mercury $1\frac{1}{2}$ hours after the operation. In this animal 0.1 mgm. of acetylcholine produced a rise in blood pressure of 102 mm.

3. *The actions of certain autonomic drugs on the vasopressor response to acetylcholine.* Whereas physostigmine (section A) renders non-effective doses of acetylcholine effective and cocaine (section D) enhances the pressor effect of acetylcholine, nicotine (12) and ergotamine (13) reverse the vasopressor effects of acetylcholine in atropinized animals.

4. *The effect of acetylcholine in the absence of sympathetic ganglia.* In each of two dogs under nembutal anesthesia and artificial respiration both superior cervical and stellate ganglia and both thoracic sympathetic chains were removed. Following this operation the chest was closed and the animals allowed to breathe again. In these animals the injection of 0.05 mgm. of acetylcholine produced marked rises in blood pressure preceded by a small fall. Following this intravenous injection of acetylcholine the abdominal aorta was clamped at the level of the diaphragm and the experiments were continued using only the anterior portion of the animal which was completely deprived of its sympathetic ganglia. Injections of 0.05 mgm. of acetylcholine into the external jugular vein now produced without exception marked falls in blood pressure. In no instance was there the slightest evidence of a nicotinic action in the absence of the sympathetic ganglia (see fig. 4).

5. *Proof of liberation of pressor substances by acetylcholine.* In seven experiments two dogs were arranged so that blood from a donor receiving 5 mgm. of atropine and 1 mgm. of physostigmine could be rapidly transferred into a cocainized recipient (5 to 10 mgm. cocaine hydrochloride, intramuscularly). In each case the donor received 0.05 to 0.1 mgm. of acetylcholine; at the beginning of the pressor response 20 cc. of blood (in one animal 8 cc.) were taken and transferred into the recipient. This quantity of blood if injected immediately into the recipient produced pressor responses from $1\frac{1}{2}$ to 6 times the rise obtained from control blood injections. The same results were obtained in one experiment with cats in which 5 cc. of blood were transferred. In an experiment on dogs, in
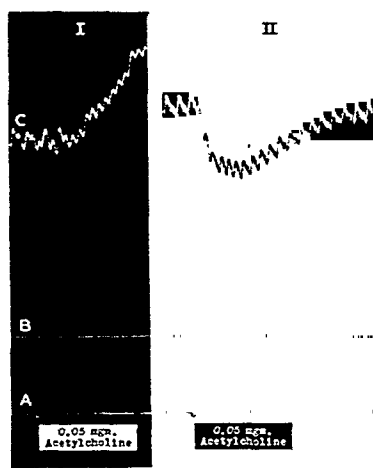
Fig. 4. Dog, 8.6 kgm., complete sympathetic gangliectomy of the upper animal (both superior cervical ganglia and both thoracic chains removed), 30 mgm. of sodium pentobarbital, 3 mgm. of atropine sulfate and 1.0 mgm. of physostigmine salicylate per kilogram, vein.

A, line indicating injection of drugs. B, base line representing zero mm. Hg pressure. C, blood pressure tracing from the common carotid artery.

I, before clamping the abdominal aorta; 0.05 mgm. of acetylcholine chloride, femoral vein. II, after clamping the abdominal aorta at the diaphragm; 0.05 mgm. of acetylcholine chloride, external jugular vein.

which the abdominal aorta and adrenal glands of the donor were clamped before 20 cc. of blood were transferred, similar results were obtained. The results were negative in one dog following the transfer of blood after acetylcholine injection and in all cases in which the recipient was not cocainized. In three of the above experiments after 1 to 3 mgm. of nicotine were administered to the donor receiving atropine and physostigmine and transfer of blood was made following acetylcholine injection, no rise in blood pressure occurred in the recipient, whereas before nicotine administration to the donor definite pressor effects were obtained upon transfer of blood following acetylcholine·injection into the donor.

In every case acetylcholine administered to the recipient produced

marked falls in blood pressure. Occasionally a rise in blood pressure followed by a secondary fall in the recipient occurred when transfer of blood was made following acetylcholine to the donor, particularly when the blood was taken too soon after acetylcholine injection or after a larger dose of acetylcholine. If the recipient received physostigmine in addition to cocaine, the transfer of blood following acetylcholine injection to the donor resulted in blood pressure falls in the recipient.

In seven dogs receiving amytal or nembutal anesthesia, it was found that the "apparent" blood epinephrine content ranged from 0.038 to 0.061 microgram per cubic centimeter as determined by Shaw's method (14) for the estimation of epinephrine. In these animals the "apparent" blood epinephrine concentration was not changed by the injection of 5 mgm. of atropine and 1 mgm. of physostigmine, the bloods being tested one-half to one hour after injection of these drugs. Blood taken at the beginning of the marked pressor responses to 0.05 to 0.1 mgm. of acetylcholine in these animals showed no alteration in the "apparent" blood epinephrine content. This indicates either that epinephrine is not liberated by acetylcholine injections or that the test is not sensitive enough to detect the increase. On the other hand, the "apparent" blood epinephrine content was increased 30 per cent in one dog (weight 6.8 kgm.) by the intravenous injection of a total dose of 0.25 cc. of 1:10,000 epinephrine solution, and in another dog (weight 7.3 kgm.) 334 per cent by a total dose of 0.25 cc. of 1:1000 epinephrine. The blood samples were taken from the femoral artery during an interval of 2 to 11 seconds after injection into the femoral vein on the opposite leg.

DISCUSSION. The ablation of the carotid sinuses and the destruction of the entire central nervous system does not interfere with the acetylcholine pressor effect in atropine-physostigminized animals. It was further demonstrated that mobilization of epinephrine from the suprarenal glands and adrenergic substances from the liver or splanchnic viscera by acetylcholine were not singly responsible for the elicitation of the acetylcholine pressor response. Sympathomimetic substances which are liberated following acetylcholine injections produce definite physiological responses when transferred from one animal to another. This substance (or substances) produced through ganglionic stimulation by acetylcholine has many epinephrine-like actions. Chemical tests indicate that this sympathetic substance, or at least considerable portions of it, is not identical with epinephrine.

It is generally admitted that the splanchnic area and the skin contain most of the effectors on which adrenergic agents act in producing vasoconstriction. In the experiments in which the splanchnic viscera were excluded by clamping the abdominal aorta and the skin removed from the

anterior part of the animal, these effectors were excluded. According to expectations in these animals, though atropinized, acetylcholine produced only its inherent vasodilator effect.

The data reported in this paper establish the fact that the "nicotine-like" action of acetylcholine is due to stimulation of vasomotor sympathetic ganglionic cells. It was shown that nicotine abolishes and reverses the pressor effect of acetylcholine in intact atropinized or atropine-physostigminized animals, both before and after the clamping of the abdominal aorta. Similar reversals of the acetylcholine pressor effects were obtained upon clamping the abdominal aorta in animals in which the cervical and thoracic sympathetic ganglia were removed. This method of complete sympathetic gangliectomy is of interest because it can be carried out fairly rapidly in acute experiments and because it is a feasible method of removing or excluding all sympathetic ganglia with abolition of all "nicotine-like" actions of acetylcholine. Bacq (15) reported that he removed all sympathetic ganglia or sectioned all sympathetic postganglionic fibers and claimed that in atropinized animals following sympathectomy the intravenous injection of 5 mgm. of acetylcholine did not produce a contraction of the muscle of the nictitating membrane. Feldberg and Minz (9) attempted partial sympathetic gangliectomies in cats and found that removal of the stellate ganglia diminished the cardio-accelerator action of acetylcholine and that the removal of the solar plexus and of the inferior mesenteric ganglia diminished the splanchnic vasoconstriction produced by acetylcholine. These authors, however, admitted that they were unable to perform complete sympathetic gangliectomies and to abolish or reverse the pressor action of acetylcholine.

The threshold of the sympathetic ganglia to acetylcholine is much higher than that of the vasodilator receptors and in most animals at least 0.5 mgm. of acetylcholine is necessary to elicit the "nicotine-like" effects. It was found that the amount of the acetylcholine sufficient to stimulate the ganglia was considerably lowered by the administration of physostigmine or prostigmin. The fact that physostigmine potentiates markedly the pressor effects of acetylcholine, but not those of muscarine and doryl, indicates that at least in part the physostigmine potentiation of the acetylcholine pressor effect is probably due to its anti-cholinesterase activity.

Atropine interferes only with the "muscarine-like" actions of acetylcholine but its opposition to these latter effects is a relative and variable one. For example, large doses of acetylcholine may produce in the presence of small or moderate amounts of atropine a rise in blood pressure, whereas a smaller dose of acetylcholine may produce a fall. The small dose of acetylcholine does not reach the threshold of the vasomotor

sympathetic ganglia, while with the large doses the resultant pressor response is a mere algebraic addition of the nicotinic and muscarinic actions of acetylcholine.

Cocaine alone does not initiate vasopressor responses to small ineffective amounts of acetylcholine in the presence of atropine, but potentiates such responses only if acetylcholine is given in doses sufficient to produce small pressor effects. These experiments show that cocaine potentiates the pressor effect of substances liberated by acetylcholine stimulation of the sympathetic ganglia.

SUMMARY

1. The anti-cholinesterases, physostigmine and prostigmin, potentiate the pressor effect of acetylcholine but not that of muscarine and doryl.

2. Small doses of acetylcholine in the presence of physostigmine or prostigmin also produce acceleration of the heart, relaxation of the gut, stimulation of the ocular sympathetics, incoördinated somatic motor efforts and stimulation of respiration in atropinized animals.

3. The pressor effects produced by acetylcholine are preserved following removal of the carotid sinuses, the central nervous system, the adrenal glands, the liver, and/or clamping of the abdominal aorta at the level of the diaphragm. Upon the removal of the thoracic sympathetic chain and the superior cervical ganglia, acetylcholine does not produce pressor effects or other nicotinic actions following clamping of the abdominal aorta. Thus the site of the pressor effect of acetylcholine has been definitely localized in the sympathetic ganglia. Purely pharmacological evidence supports this conclusion (nicotine, a ganglionic paralysant, abolishes and often reverses the pressor effect of acetylcholine).

4. The substance or substances liberated by acetylcholine stimulation of the sympathetic ganglia may be transferred from one animal to another producing pressor effects in the recipient.

5. Cocaine does not initiate pressor responses from small ineffective doses of acetylcholine in atropinized animals, but it enhances these effects from effective doses of acetylcholine.

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ADAPTATION TO ESTROGEN OVERDOSAGE

An Acquired Hormone Resistance Without Antihormone Formation

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The fact that an animal may become resistant to the action of a hormone after a period of pretreatment has first clearly been shown for the parathyroid hormone (1). Later we made similar observations concerning the gonadotropic principles and concluded that in the latter case the resistance is due to the formation of hormone antagonistic substances since we found that "the blood of animals which have become insensitive to the anterior pituitary-like hormone of pregnancy urine is able to inactivate this hormone" (2, 3). On the basis of these experiments, Collip (4) formulated the theory that resistance to gonadotropic hormone develops because the organism produces a specific "antihormone" against this principle. Numerous other instances in which the antihormone theory of hormone resistance proved applicable have recently been considered in our detailed review of this subject (5). It was observed, however, that in many cases, hormone resistance may develop without the formation of demonstrable antihormones. Thus we consistently failed to reveal any parathyroid hormone neutralizing effect in the blood of animals pretreated with this principle (6). Similarly we showed that although chronic treatment with adrenalin fails to elicit the formation of a specific adrenalin antagonizing principle, it gives the organism a very marked resistance against this hormone (7, 8). We noted, furthermore that the enlargement of the pituitary, the adrenal cortex and the corpora lutea elicited in the rat by estrogen overdosage regresses after several weeks of treatment in spite of daily estrogen administration. We had to conclude therefore, that the organism may acquire estrogen resistance (9) although others have shown (10–12) and we may confirm (6) that estrin does not lead to antihormone formation. More recently we noted that young rats chronically treated with large doses of estrogens show a marked decrease in body weight during the first week but then reveal the development of resistance by the resumption of normal growth and weight increase. This readily distinguishable external sign of adaptation to the systemic effects of estrogen overdosage proved to be a useful indicator which served

as a basis for the experiments to be reported in this communication. The main purpose of these experiments was to analyze further the phenomenon of hormone resistance without antihormone formation and to establish the degree of specificity of such a resistance. As will be seen from the experiments reported in the experimental part of this paper, this resistance is not very specific inasmuch as pretreatment with estradiol renders animals resistant to the artificial synthetic estrogen diethylstilbestrol and *vice versa*.

EXPERIMENTAL PART. In our first experiment, we used 32 male and 32 female growing albino rats. At the beginning of the experiment the body weight of the males varied between 110 and 143 grams and that of the females between 107 and 145 grams. Eight males and 8 females received daily doses of 2 mgm. of estradiol subcutaneously in 0.1 cc. of peanut oil while 8 males and 8 females were given subcutaneous injections of the same
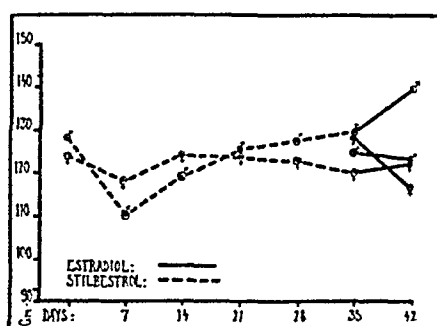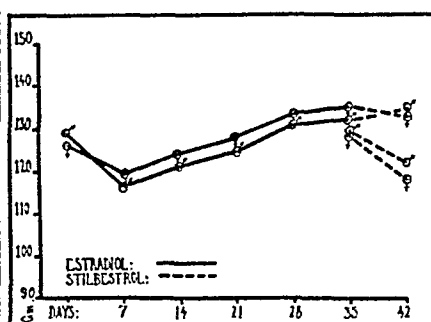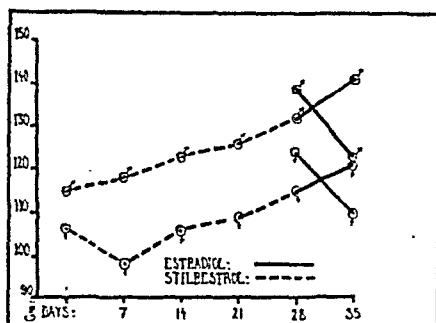

Fig. 1   Fig. 2
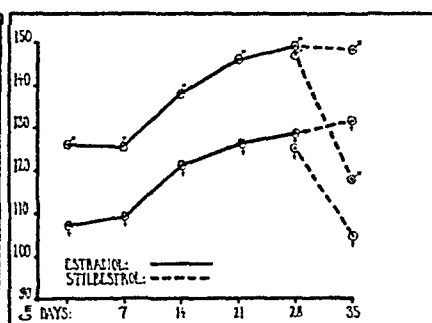
Fig. 1. Weight curves of rats adapted to a large dose of stilbestrol, then treated with estradiol and of controls not adapted receiving estradiol during the last week of the experiment.

Fig. 2. Weight curves of rats adapted to a large dose of estradiol, then treated with stilbestrol and of controls not adapted receiving stilbestrol during the last week of the experiment.

daily dose of 4:4′-dihydroxy-α:β-diethylstilbene (diethylstilbestrol or stilbestrol) dissolved in the same amount of peanut oil. The animals were weighed at the onset of the experiment and then once a week throughout the treatment period. From their average weight, we constructed the growth curves shown in figures 1 and 2. These graphs indicate that during the first week, the rats of both sexes lost a great deal of weight as a result of the estrin treatment. By the end of the second week their weight had begun to increase, however, and in spite of continued daily treatment with the estrogens, the animals in all groups except in that of the diethylstilbestrol treated females continued to gain until the fifth week. In the latter group, after the recovery from the initial weight loss, the body weight remained fairly constant. It is noteworthy that although before treatment, the males of both experimental series were larger than

the females, the former lost so much more weight during the initial phase
of estrogen administration that by the end of the first week, their average
weight was below that of the females. At the end of the fifth week, the
treatment of the animals adapted to estradiol was changed to diethyl-
stilbestrol, while the animals which had so far been treated with the latter
compound were given the same amount of estradiol for one week. As
the graph indicates, this change in the type of the estrogen administered
did not lead to any significant difference in the growth rate. At the same
time, that is to say, during the fifth week of the experiment two other
groups each consisting of 8 male and 8 female rats—which had not been
pretreated but were approximately comparable in size to the estrogen
pretreated animals—were given similar daily injections of estradiol and
diethylstilbestrol respectively. The graph shows the considerable loss
of weight which resulted from this treatment, a loss of weight which is
approximately similar to that occasioned by the estrogens in the pre-
treated group during the first week of treatment. This experiment indi-
cates quite clearly that resistance may be acquired to the toxic actions
of both estradiol and diethylstilbestrol and that pretreatment with one
of these estrogens renders the animals resistant to both estrogenic com-
pounds.

It appeared of interest to establish whether a similar resistance to the
toxic effect of large doses of estrogen may also be obtained by pretreat-
ment with smaller doses. In order to determine this and to obtain further
evidence supporting the conception of "crossed resistance" between the
naturally occurring and artificial estrogens, we performed the following
experiments.

Sixteen male (body weight 100–129 grams) and 16 female rats (body
weight 96–120 grams) were divided into two groups, each of which con-
tained 8 males and 8 females. One group received daily injections of
300 gamma of diethylstilbestrol and the other 300 gamma of estradiol
subcutaneously in 0.1 cc. of peanut oil daily. Figures 3 and 4 show that
this dose was tolerated without any loss of weight by the diethylstilbestrol
treated males and the estradiol treated females while the remaining two
groups showed a slight initial decrease in the average body weight. How-
ever growth was soon resumed by all groups and at the end of the fourth
week, the body weight was far above the initial value in all animals. At
this time, the estradiol pretreated rats were changed to daily injections of
1 mgm. of diethylstilbestrol while the animals pretreated with the latter
substance received 1 mgm. of estradiol. Both substances were ad-
ministered subcutaneously in 0.1 cc. of peanut oil. As indicated by our
graphs, the trend of the growth curves was not significantly altered by this
change to a large dose of a different estrogen. At the end of the fourth
week, that is to say, at the time when the change in treatment occurred, we

began injecting a group of 8 males and 8 females with similar 1 mgm. daily doses of diethylstilbestrol and another group of 8 males and 8 females with the same amount of estradiol. These animals which had not been pretreated with estrogens showed a considerable decrease in body weight as indicated in figures 3 and 4, thus demonstrating that the pretreated group has really acquired a definite estrogen insensitivity.

DISCUSSION. Our experiments indicate that the organism may adapt itself at least to the toxic effects of both estradiol and stilbestrol. While this is merely a confirmation and extension of previous observations (9, 13–15), it is of particular interest that adaptation to the naturally occurring steroid hormone, estradiol, induces resistance to a stilbene derivative which has a different chemical structure. At least in this instance, it appears that acquired hormone resistance without antihormone

Fig. 3   Fig. 4

Fig. 3. Weight curves of rats adapted to a relatively small dose of stilbestrol, then treated with a large dose of estradiol and of controls not adapted receiving estradiol during the last week of the experiment.

Fig. 4. Weight curves of rats adapted to a relatively small dose of estradiol, then treated with a large dose of stilbestrol and of controls not adapted receiving stilbestrol during the last week of the experiment.

formation is less specific than it usually is in cases of antihormone production. It will be recalled that resistance to gonadotropic preparations from the pituitary which is due to antihormone formation does not render animals insensitive to gonadotropic pregnancy urine extracts or *vice versa* (2, 3). Furthermore, antihormones elicited by treatment with a certain thyrotropic preparation have little or no effect on thyrotropic extracts prepared by a different method (16, 17).

With regard to the theoretical interpretation of the mechanism responsible for the crossed resistance between the two estrogens which we used, the following two possibilities present themselves. First it is conceivable that diethylstilbestrol is transformed in the organism to a substance similar to or identical in structure with estradiol and that it acts only after transformation into a compound of the steroid type. If this were

true, we would not be dealing with a real example of crossed resistance, since at the time when the estrogens which we employed exerted their pharmacological action, they would have become chemically identical or at least extremely similar. However, this interpretation receives no support from the bioassays which demonstrate that weight for weight, diethylstilbestrol is more active than most of the natural estrogens. The findings of Stroud (18) likewise indicate that the assumption of such a transformation is unfounded since he showed that diethylstilbestrol may be recovered as such from the urine of rabbits treated with this compound. It is true that the recovery is not quantitative but the author states that "... 4:4'-dihydroxy-α:β-diethylstilbene give recoveries of the order of 20 per cent compared with 1.5 per cent found for oestrone. This indicates a metabolic process for the synthetic oestrogens different from that of oestrone." These observations are not readily compatible with the view that diethylstilbestrol acts only after conversion into a steroid estrogen similar to the naturally occurring compounds.

A second and more likely explanation of our findings is that the organism does not become resistant to a certain chemical substance with estrogenic actions (that is to say, in our case to estradiol or diethylstilbestrol) as such, but to the estrogenic effect itself. If such an interpretation should prove to be correct, this case of crossed resistance would deserve special interest inasmuch as it would be a case of *adaptation to an action or effect rather than to a certain chemical substance or drug.*

Before closing this discussion, we should like to add that in numerous experimental series we noted that with very long continued estrone, estradiol or diethylstilbestrol treatment in doses of 2 to 5 mgm. daily, rats weighing approximately 100–150 grams at the onset of the experiment show the usual loss of weight during the first week followed by adaptation and increase in weight during the subsequent 5 to 6 weeks which is in accord with what has been said above. In these very chronic experiments however, where treatment was continued for 2 to 3 months, we invariably observed that after the period of adaptation, a third stage follows during which the acquired resistance appears to vanish so that the animals begin to lose weight again just as they did before adaptation occurred. We are not reporting these experiments in detail mainly because, unlike the initial period of weight loss and the period of adaptation, the final exhaustion of the adaptive mechanism and loss of weight is subject to great individual variations, one animal of a group beginning to lose weight after 5 or 6 weeks while another may go on for three months before its weight curve declines. Because of this great variability, each animal would have to be considered separately and since little would be gained from the study of such long individual weight curves or tables, we are omitting them here for the sake of brevity. Yet we want to mention these observations because they confirm our contention that the syndrome of adaptation to

estrogens is similar to that which develops during adaptation to various other damaging agents. It has been shown that during adaptation to almost any change in the internal or external environment of the organism, a so-called "general adaptation syndrome" is elicited which has three distinct stages. These have been termed the "stage of the alarm reaction," the "stage of resistance" and the "stage of exhaustion" respectively. During the first stage, among other symptoms and signs there is considerable loss of body weight, adrenal enlargement, involution of the lymphatic organs and water retention. During the second stage, most of these changes disappear and the animals become very resistant to the agent with which they are treated. Eventually however, during the third stage they again begin to lose weight and finally die with symptoms and signs similar to those seen during the initial stage. On the basis of these observations, we assumed that there is a general non-specific adaptation mechanism which facilitates the acquisition of resistance against a great many agents and that in case of prolonged exposure, this mechanism wears out and the adaptability or "adaptation energy" becomes exhausted (8). The literature on this adaptation syndrome has recently been reviewed by Leblond (18) and Varangot (19). Selye et al. (20) and Selye (21) showed that acute overdosage with estrogens may lead to a typical "alarm reaction" and the present experiments indicate that under the influence of continued treatment with estrogenic substances a "stage of resistance" and finally a "stage of exhaustion" develops. From this we may conclude that even adaptation to estrogens is not permanent but subject to the general law of the eventual exhaustion of adaptation energy as outlined in the above mentioned publications.

SUMMARY

Experiments indicate that the weight of growing rats chronically treated with estrogens first declines but growth is later resumed and the body weight may actually exceed the initial level in spite of continued administration of massive doses of estradiol or diethylstilbestrol. This is interpreted as additional evidence indicating that adaptation is possible at least to the toxic action of these estrogens although they do not elicit antihormone formation.

Rats adapted to the natural estrogen, estradiol, prove to be equally resistant to the artificial estrogenic compound, diethylstilbestrol, although chemically the latter is quite different from the former. Conversely diethylstilbestrol pretreatment renders the rat resistant to the toxic actions of estradiol. A review of the literature gives no support to the assumption that this "crossed resistance" should be regarded as due to the transformation of diethylstilbestrol into a naturally occurring estrogen similar to or identical with estradiol. It is concluded therefore, that the most probable interpretation of these findings is that we are dealing with a case of

acquired resistance to a certain (estrogenic) pharmacological action rather than to a particular chemical substance.

In case of very prolonged daily administration of relatively large doses of estrogens, the acquired adaptation is gradually lost. The animals, which following an initial stage of weight loss had adapted themselves to the treatment sufficiently to gain weight in spite of continued treatment, eventually lose weight again and finally die after two or three months of estrogen administration. It is concluded that adaptation to the toxic actions of estrogens takes place in the same manner as adaptation to most noxious agents, namely, by the development of the "general adaptation syndrome" with its characteristic three stages. *The eventual loss of an already acquired adaptation to estrogens gives further support to the conception that the adaptability or "adaptation energy" of the organism is a limited quantity and is gradually consumed while the organism puts up resistance against a stimulus to which it appears to be adapted.*

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THE ESSENTIAL NATURE OF PANTOTHENIC ACID AND ANOTHER ALKALI LABILE FACTOR IN THE NUTRITION OF THE DOG[1,2,3]

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In a previous publication (1) evidence was presented for the necessity of a factor separate from thiamin, riboflavin, nicotinic acid, and vitamin B_6 in the nutrition of the dog. This factor followed factor W in all fractionation·procedures and we suggested the identity of these two factors. Since pantothenic acid, the chick antidermatitis factor, has been shown in several laboratories (2, 3, 4) to be essential for the rat, it seemed advisable to investigate its importance for the dog. The results obtained with the liver extract fractions reported previously were not entirely suited to a study of pantothenic acid, and it was decided to make a more comprehensive investigation of this problem. In this paper we wish to show that in addition to factor W, dogs require pantothenic acid and another alkali labile factor.

EXPERIMENTAL. Newly weaned puppies and older growing dogs were used in all of the experiments and the general experimental procedure was very similar to that employed in our earlier studies. Thiamin, riboflavin and nicotinic acid were supplied as previously described. In addition pure vitamin B_6 in aqueous-alcoholic solution was given orally twice weekly at a level of 60 micrograms per kilogram of body weight per day.[5]

The known chemical properties of pantothenic acid suggested two methods of removing it from growth promoting fractions of liver extract: 1, destruction with alkali, and 2, extraction with ether at acid pH. The assay method for pantothenic acid used in all of this work is that described

by Snell et al. (5, 6). It has been the experience of this laboratory that this method of assay correlates very well with the method for the chick antidermatitis factor using chicks.

Alkali inactivation. It was found that heating the hexane-butanol extract (7) on the steam bath (87–93°C.) with KOH at a concentration of 0.2 N for two hours completely destroyed the pantothenic acid present. Since the hexane-butanol extract allowed good growth when supplemented with vitamin B_6, it was thought that the alkali inactivated extract might furnish a suitable vitamin B complex source free of pantothenic acid. For a source of pantothenic acid a concentrate prepared by Wilson Laboratories was used throughout the experiment.[6] This concentrate was prepared from liver extract according to the method of Woolley et al. (8). It contained 3.5 milligrams of pantothenic acid per cc. and on the solid

TABLE 1

Growth response of young puppies on pantothenic acid and pantothenic acid-free concentrates

DOG NUMBER	LIVER FRACTION	TEST PERIOD	INITIAL WEIGHT	TOTAL GROWTH	GROWTH PER DAY	REMARKS
		days	*kgm.*	*kgm.*	*kgm.*	
1	3% hexane butanol extract	42	2.45	2.125	0.051	Excellent growth
2	14.6 mgm. Wilson's pantothenic acid over assay period	18	2.125	0.375	0.021	Poor growth
2	10 mgm. Wilson's pantothenic acid + 3% alkali inact. hexane-butanol ext.	27	2.500	1.075	0.040	Good growth
3	4% alkali inact. hexane butanol ext.	16	2.70	0.725	0.045	Good growth but dog died suddenly

basis contained 3.5 per cent pantothenic acid. An ether extract of this concentrate at acid pH was also found to be suitable as a source of pantothenic acid, and this preparation was also used in some of the trials.

A litter of three puppies was then placed on experiment for a study of the response to these fractions. One received in addition to the four crystalline vitamins the original hexane-butanol extract at a level of 3 per cent; another received the alkali inactivated hexane-butanol extract at a level equivalent to 4 per cent; while the third received the Wilson Laboratories pantothenic acid concentrate at a level equivalent to about 500 micrograms of pantothenic acid per day. The response of these dogs is given in table 1. The positive control showed an excellent rate of

growth but the dog receiving only pantothenic acid grew very poorly. When the pantothenic acid preparation was supplemented with the alkali inactivated hexane-butanol extract a marked increase in the rate of growth resulted. The dog receiving only the alkali inactivated extract grew rapidly for 16 days and then died very suddenly. The autopsy revealed no evidence of infection of any kind nor any other obvious cause of death. The failure was undoubtedly nutritional.

To continue the study six older dogs were placed on the basal ration containing the four pure vitamins. All of these dogs showed a growth plateau followed by anorexia and a marked loss of weight. Definite growth responses resulted when the pantothenic acid concentrate or the acid ether extract of this concentrate was injected. If, however, the animal was maintained on this ration continuously after one or several responses to pantothenic acid, a point was always reached when no response could be elicited by pantothenic acid. This was also the case when the alkali inactivated hexane-butanol extract was included in the ration at a level of 3 per cent. Addition of whole liver extract or the alcohol ether precipitate fraction produced an immediate recovery, but the alcohol ether filtrate and high levels of the alkali treated hexane-butanol extract were ineffective. Typical results obtained with these dogs are shown in figure 1.

Ether extraction. The removal of pantothenic acid from liver extract was accomplished with the following procedure: 200 grams alcohol soluble liver extract no. 39796 was diluted to one liter with water and 1:1 H_2SO_4 added until the pH was approximately 2.0. The precipitate thus formed was filtered off and the filtrate was placed in a continuous liquid-to-liquid extractor and extracted with peroxide free ether for 5 to 7 days. The pantothenic acid content of this ether residue was found to be not greater than 14 micrograms per gram of original liver extract.

This fraction was then added to the basal ration at a level of 2 per cent. Several older growing dogs were used for this study, all of which had been on fractions supplying large amounts of pantothenic acid. On this fraction dog 7 gained almost 4 kgm. in 80 days, then a plateau in the rate of growth occurred followed by anorexia and diarrhea with a consequent loss of weight. At this point injection of 3.5 cc. of a pantothenic acid concentrate produced an immediate remission from this condition as shown in figure 1. The pantothenic acid concentrate was prepared from the alcohol ether filtrate fraction of liver extract powder followed by adsorption and elution of the vitamin from active norite as was employed in that concentrate prepared by the Wilson Laboratories. The eluate was finally extracted with ether at acid pH. This extract contained only 1.85 per cent solids of which 5.4 per cent was pantothenic acid. Several days after the peak of growth response was reached the animal was found in a deep coma and died a few hours later. The comatose state was characterized

by bradycardia, deep labored breathing, and a temperature of 105 degrees. This syndrome simulated in many respects that previously reported for dog 14 (1). Another dog on this residue for 88 days was found in a
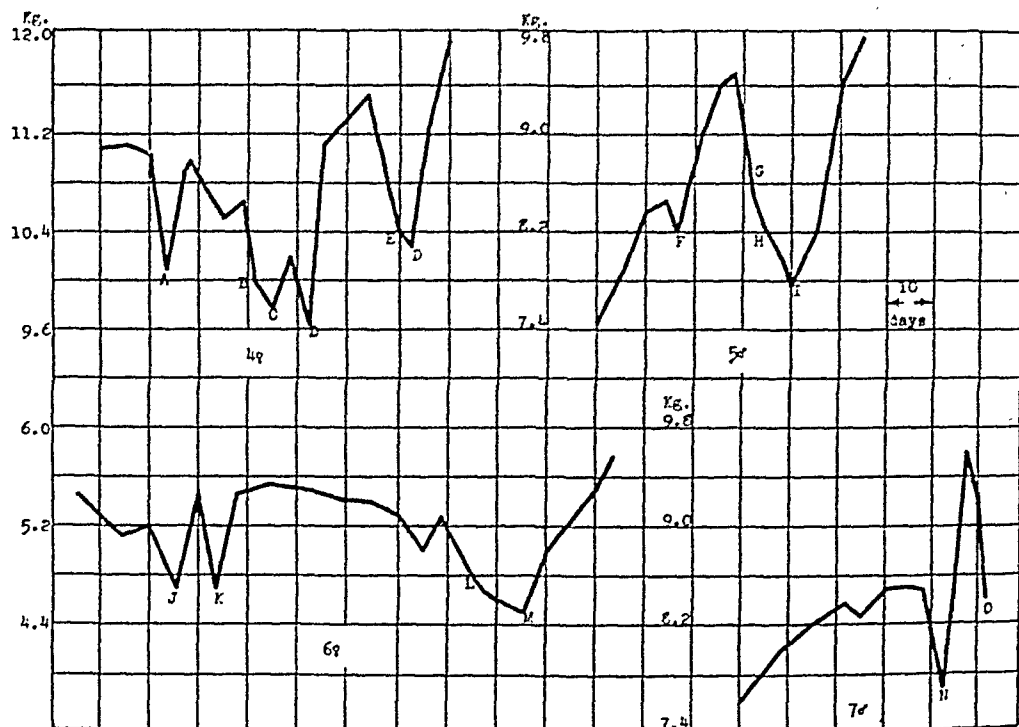
Fig. 1. Growth curves of older dogs on pantothenic acid deficient rations.

Dog 4—Basal ration. A, Wilson pantothenic acid concentrate equivalent to 20 mgm. pantothenic acid. B, Wilson pantothenic acid concentrate equivalent to 20.7 mgm. pantothenic acid. C, alkali treated hexane-butanol extract equivalent to 52 grams liver extract + 4 per cent on ration. D, 2 per cent alcohol ether precipitate on ration. E, Wilson pantothenic acid concentrate equivalent to 21 mgm. pantothenic acid.

Dog 5—Basal ration. F, Wilson pantothenic acid concentrate equivalent to 17.5 mgm. pantothenic acid. G, Wilson pantothenic acid concentrate equivalent to 45 mgm. pantothenic acid. H, alkali treated hexane-butanol extract equivalent to 80 grams liver extract + 5 per cent on ration. I, started 4 per cent liver extract powder on ration.

Dog 6—3 per cent alkali treated hexane-butanol extract on ration. J, Wilson pantothenic acid concentrate equivalent to 7.13 mgm. pantothenic acid. K, Wilson pantothenic acid concentrate equivalent to 14.0 mgm. pantothenic acid. L, Wilson pantothenic acid concentrate equivalent to 23.8 mgm. pantothenic acid. M, started 4 per cent liver extract 39796 on ration + 20 grams liver extract powder orally.

Dog 7—2 per cent acid ether residue. N, pantothenic acid concentrate equivalent to 8 mgm. pantothenic acid. O, animal died.

similar comatose condition and died shortly after. Autopsy of both dogs revealed mottled livers, severe gastritis particularly of the pylorus, and heavy bile pigmentation throughout the intestine.

To investigate further the use of ether residues in producing the deficiency a litter of six young puppies was placed on the basal ration supplemented with the four crystalline vitamins. Two were given the acid ether residue at a level of 2 per cent, two were given the acid ether extract at a level of 2 per cent, and the last two were given molasses at a level of 6 per cent. This molasses was found to be a good source of factor W accord-
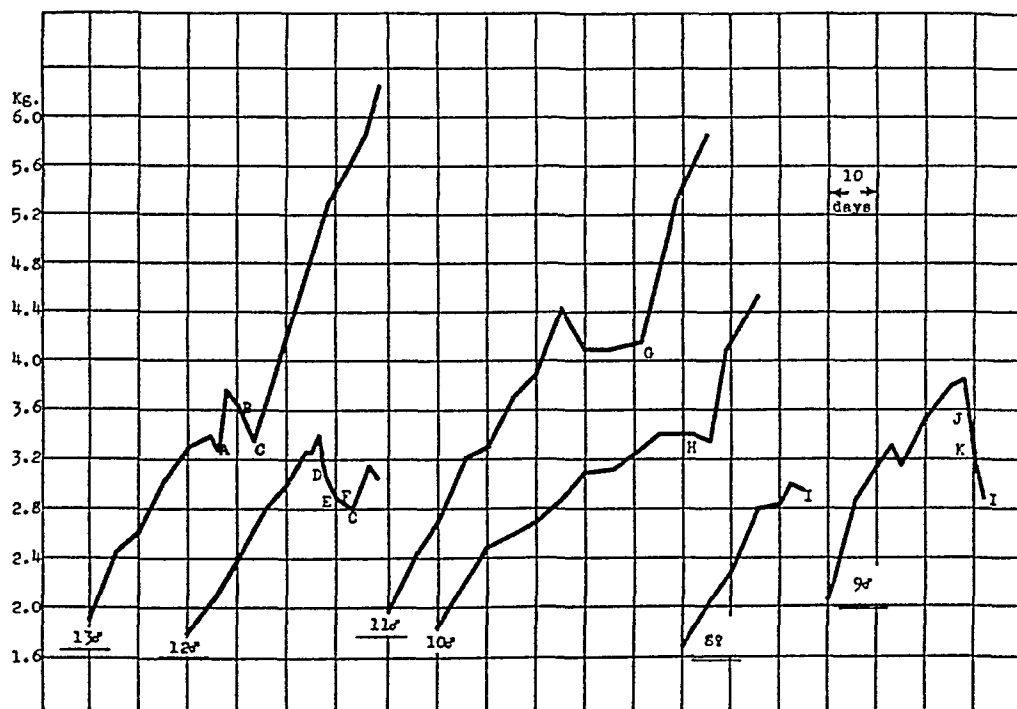
Fig. 2. Growth curves of young puppies on pantothenic acid and factor W deficient rations.

Dogs 12 and 13—6 per cent molasses. A, pantothenic acid concentrate equivalent to 1.89 mgm. pantothenic acid. B, acid ether extract equivalent to 25 grams liver extract. C, changed to 4 per cent liver extract powder. D, pantothenic acid concentrate equivalent to 3.8 mgm. pantothenic acid. E, 20 mgm. choline hydrochloride. F, acid ether extract equivalent to 22.5 gram liver extract.

Dogs 10 and 11—2 per cent acid ether extract. G, changed to 2 per cent alkali treated acid ether residue. H, changed to 2 per cent acid ether residue.

Dogs 8 and 9—2 per cent acid ether residue. I, animal died. J, pantothenic acid concentrate equivalent to 4 mgm. pantothenic acid. K, acid ether extract equivalent to 30 grams liver extract.

ing to the rat assay method and contained only 7 micrograms of pantothenic acid per gram. The growth response of these dogs is given in figure 2.

The two dogs receiving the acid ether residue grew very well for 4 weeks and then failed very suddenly. An injection of the pantothenic acid concentrate was given to dog 9 but no beneficial effects were observed. Upon

autopsy an intussusception was found in the lower small intestine which explains the anorexia and frequent vomiting movements as well as the primary cause of death. Light colored livers were found in both dogs. The yellowest was assayed for its riboflavin content by the method of Snell and Strong (9) and was found to contain 32 micrograms per gram of wet liver.

The two dogs receiving the acid ether extract grew fairly well but eventually plateaued after 7 weeks. Replacement of the extract with the acid ether residue or the alkali treated acid ether residue at a level of 2 per cent produced immediate responses in both cases. The latter fraction was prepared by diluting the acid ether residue 10 times with water, adding solid KOH to a concentration of 1 normal and then heating on the steam bath at 85–88°C. for $1\frac{1}{4}$ hours. The dogs receiving molasses grew very well for 4 weeks and then started to decline. Dog 12 failed to respond to pantothenic acid, choline, or the acid ether extract, but did show improvement for a time on liver extract powder until a serious infection incurred at the point of injection of the liver fractions. Dog 13 showed a slight response to the pantothenic acid concentrate, but not to a subsequent dose given as the acid ether extract fraction. The addition of 3 per cent liver extract powder to the basal ration resulted in an immediate recovery.

DISCUSSION. It is apparent from the data presented above that the dog requires, in addition to factor W (the alkali stable factor), two essential factors which are destroyed by alkali. Thus dog 6 receiving the alkali treated hexane-butanol extract responded at first to injections of the pantothenic acid concentrate. Maintained on this fraction the animal became deficient again and would not respond to injections of much larger doses of pantothenic acid. Liver extract powder was required to restore his weight back to the original level. This was also true in the case of dog 5. After an initial response to pantothenic acid a relapse occurred which could not be cured by a combination of 45 mgm. of pantothenic acid in concentrate form and 80 grams of the alkali treated hexane-butanol extract given orally and parenterally as well as an additional 5 per cent of this fraction on the ration. Four per cent of liver extract powder supplemented to the basal ration completely restored the animal to normal.

The immediate temporary response of all dogs to injections of the highly purified pantothenic acid concentrates indicates strongly that this factor is required by the dog. The striking remission in the case of dog 7 was obtained on the pantothenic acid concentrate which represented only 17.5 grams of liver extract powder. It is unlikely that any factor other than pantothenic acid should follow so closely and so quantitatively. However, the matter cannot be established for certain until pure pantothenic acid is available.

The differentiation between pantothenic acid and the other essential

factors is further shown by the behavior of dog 4. Left on the basal ration alone the animal failed to respond a second time to 20 mgm. pantothenic acid and to large quantities of the alkali treated hexane-butanol extract. However, the addition of the alcohol ether precipitate at a 2 per cent level resulted in immediate recovery. This fraction contains no more than 20 micrograms of pantothenic acid per gram equivalent of liver extract powder. The recovery on such a fraction proves that there existed a deficiency of other factors.

Our experience with the acid ether fractions indicates that acid ether extraction removes other essential factors as well as pantothenic acid. The survival obtained with the acid ether extract alone at 2 per cent indicates that this fraction carries appreciable amounts of the alkali labile factor and just enough factor W for maintenance. The substitution of the alkali treated residue at a level of 2 per cent produced an excellent growth response further demonstrating the essential nature of the alkali stable factor W. The collapse of dog 7 could be ascribed to the inadequacy of the acid ether residue in factors other than pantothenic acid, yet the possibility of another acute pantothenic acid deficiency brought on by the rapid growth should also be considered.

The failure of dogs 12 and 13 to be maintained on molasses when supplemented with pantothenic acid indicates that molasses is also unsatisfactory as a B complex source for a study of pantothenic acid deficiency. Since molasses is adequate as a factor W source at 4 per cent for rat growth, it is probable that the dog requires still another factor. Recovery from this deficiency was obtained with 3 per cent liver extract in both cases although infection seriously hampered dog 12.

The complicated nature of the pantothenic acid deficiencies observed thus far reduces the significance of the variety of symptoms which we have seen. Irregular cardiac activity in tachycardia, less frequently in bradycardia, nausea and vomiting, intussusception, and finally loss of fur and dry scaliness of the skin suggest an impairment of autonomic control and especially that of the parasympathetic nervous system. What factor or factors these symptoms are attributable to cannot be seen in the light of present evidence.

SUMMARY

1. Evidence is presented for the necessity of pantothenic acid in addition to factor W in the nutrition of the dog. Dogs fail on alkali inactivated or acid ether extracted liver extract preparations and show a growth response upon the addition of concentrates of pantothenic acid. The rations employed thus far are not suitable for study of an uncomplicated pantothenic acid deficiency.

2. Dogs receiving the alkali treated factor W preparation and the

pantothenic acid concentrate do not show normal growth until other fractions are added to the ration. It is evident that there is an alkali labile factor other than pantothenic acid which is essential for the dog.

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FACTORS AFFECTING THE MAINTENANCE OF COBALT POLYCYTHEMIA IN THE RAT[1]

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In the studies on cobalt polycythemia in this laboratory the observation has frequently been made that rats failed to maintain high levels of polycythemia. The level of hemoglobin and erythrocytes showed significant decreases, returning in some cases to normal. Subsequently the polycythemia would develop again, but it was difficult to maintain a high level of polycythemia for any length of time without these rather marked fluctuations.

The animals were receiving 0.03 mgm. of manganese per day which is considerably less than the level used by Orten et al. (1), but since these investigators have only reported studies on two relatively high levels (1.0 and 5.0 mgm. per day) no data were available which would indicate the level of manganese necessary for the stabilizing effect which they observed. The level of manganese which was employed in this study would be adequate for other known requirements hence other possible explanations for these effects were sought.

Since these animals were subjected to an abnormal hematopoietic stress it was possible that milk might not be sufficient in all respects as a basal food. Marshall (2) studying the effects of liver administration to polycythemic rats reported that liver extract produced a temporary depression in the polycythemia, followed by an increase to a level above the initially high level. Fresh liver produced an increase in the level of polycythemia without the temporary decrease noted with the extract. Considering these observations, then, it seemed possible that liver or certain liver fractions might aid in stabilizing the high levels of polycythemia, when added to the milk ration.

In this report we wish to present the results obtained with various liver fractions as a supplement to the mineralized milk along with a modified procedure for drawing the blood samples for hemoglobin determinations.

EXPERIMENTAL. Male albino rats weighing approximately 200 to 250 grams were placed on a mineralized milk[3] ration. Hemoglobin determinations were followed as an index of polycythemia since the correlation between cell count and hemoglobin level has been well enough established (3) for the purposes of this experiment. Results of comparative determinations, however, on 12 of these animals showed that rats with a hemoglobin level of 23.2 grams per 100 cc. of blood had an erythrocyte count of approximately 11.6 million per cubic millimeter, as compared with the normal
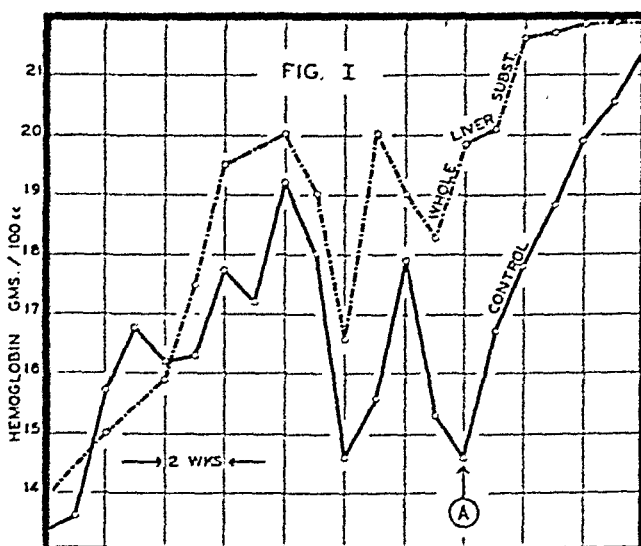
Fig. 1. Hemoglobin curves of rats receiving cobalt   Note the consistently higher level of polycythemia in the rats receiving whole liver substance. *A* indicates the point at which the new method of drawing blood was employed.

hemoglobin level of approximately 14.5 and erythrocyte count of 7.5 million.

In the first series of animals, half of the group received 1 gram of Wilson's whole liver substance per day. Figure 1 summarizes the results obtained. Although each curve represents the average of three rats, the data are typical of many more. It will be noted that in both groups the fluctuations occurred in the hemoglobin curves although the animals on the liver supplement maintained a significantly higher level of hemoglobin.

Up to this time the technique employed for drawing blood samples had

[3] Daily supplement per rat included: 0.7 mgm. of Fe as the pyrophosphate, 0.07 mgm. of Cu as the sulfate, 0.03 mgm. of Mn as the chloride, 0.7 mgm. of Co as the chloride.

included clipping the end of rat's tail. The relative degree of injury to the animal became progressively worse as the experiment proceeded and it was difficult at times to prevent excess bleeding. Bleeding could be stopped by pressure on the tip of the tail or by heat cautery, but the animals would often reopen the wound when returned to their cages. Considerable loss of blood under such conditions prompted a search for a more satisfactory method for drawing the blood sample. Various methods were tried but the most satisfactory seemed to require the use of a razor blade or sharp scalpel with which the lateral vein of the tail could be severed, without cutting through the tail. The resulting clean cut would bleed freely but could be sealed readily by slight pressure of the forefinger directly over the cut. Employing this technique no more than 5 drops of blood were required from the animal to assure free flowing blood and an adequate sample for the hemoglobin determination. Likewise the inflammation which develops around the injured portion of the tail after clipping with scissors did not appear and the general disturbance of the animal was greatly decreased.

At the point A in figure 1 this modified technique was employed in the blood sampling. The resulting smooth curve indicates that hemorrhage may have had considerable influence upon the variability of the previous hemoglobin levels.

Since the animals receiving the whole liver substance showed a consistently higher level of hemoglobin than the control group, it appeared that liver also had a significant effect in maintaining the high level of polycythemia. In the next series then the effects of whole liver substance, liver extract and liver residue,[4] were studied.

Lowering the calcium-phosphorus ratio of a ration by adding NaH_2PO_4 has been shown (4) to increase iron utilization. It seemed possible that it might likewise speed up cobalt assimilation so in one group an amount of the NaH_2PO_4 was added to the milk sufficient to reduce the Ca/P to 0.6. Casein was employed as a supplement to another group to indicate the possibility of a purely protein effect. Lilly's P. A. concentrate for oral administration was employed with another group as a further possible fractionation of the liver substance.

Figure 2 summarizes the results obtained with those groups showing significant differences. Here again only typical data are presented, each curve representing the average of 3 rats. It will be noted that the liver extract (Lilly) allowed the highest level of polycythemia. In one case an animal attained a level of 28 grams of hemoglobin per 100 cc. of blood. Whole liver substance likewise allows a more rapid induction of polycy-

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[4] These liver fractions were kindly supplied by Dr. David Klein of the Wilson Laboratories, Chicago, Illinois.

themia and the attainment of a higher level than the control animals. The NaH_2PO_4 practically eliminated the lag phase typical of the early part of the curve for the control group, but the animals exhibited the most marked symptoms of toxicity of the whole series. This observation is further substantiated by the depression of the growth curve. Of the growth curves in general it may be stated that the more rapid the production of polycythemia the more depressing the effect upon growth.
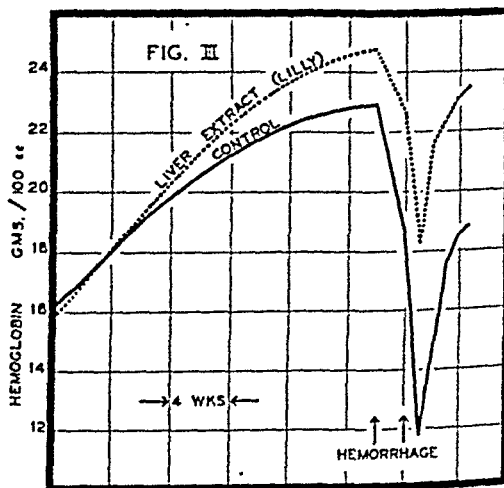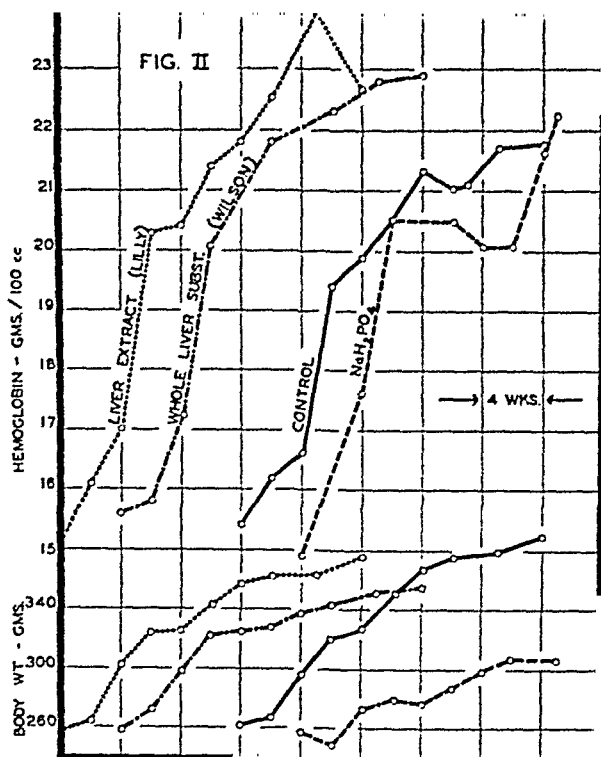
Fig. 2. Comparison of the groups of rats receiving supplements to the mineralized milk. Upper curves represent hemoglobin level, lower curves body weight. Note the higher levels of polycythemia in the animals receiving whole liver substance and liver extract. Note the rapid induction of polycythemia in the rats receiving NaH_2PO_4, with the resulting retarded growth.

Fig. 3. Hemoglobin curves of individual rats showing the effect of hemorrhage. Note the stabilizing effect of the liver extract.

The group receiving casein and the group receiving Wilson's liver extract showed only a slightly higher hemoglobin curve than the control group while the group on the liver residue showed a curve identical with the control group. Hence these curves were not included in this report.

It should be noted that the hemoglobin curves in this series are relatively smooth. Since the only difference between the groups of this series and those of the first series was the method of drawing the blood samples, it

appears that hemorrhage definitely affected the former results. As a further proof of the effect of hemorrhage, it was decided to produce a hemorrhage artificially, taking advantage of the tendency to bleed freely from a clean cut in the tail vein. In this case the tail was completely severed near the tip, and by frequently dilating the blood vessels with warm water and gently massaging the lateral veins toward the open wound, the animals could easily be bled to death. For our purposes, however, approximately 5 cc. were removed at a time and the results are shown in figure 3. The similarity between this curve and those obtained on the former animals in which hemorrhage was accidental, lends further evidence to the concept that the loss of blood was a significant factor in the failure to maintain high levels of polycythemia.

The supplemental effect of whole liver substance (Wilson) and liver extract (Lilly) was again demonstrated by the higher level of polycythemia produced, and by the fact that animals receiving the whole liver or liver extract failed to show the severe decreases in hemoglobin noted in the control group. In each case the control animal showed greater drops in the hemoglobin level although the amount of hemorrhage was equivalent.

DISCUSSION. From the data presented in this report two facts seem evident. First, the technique for drawing blood samples cannot be the same as that generally used for young rats where only 3 or 4 determinations are required. The degree of injury and the resulting loss of blood introduce undesirable results into studies on the levels of blood constituents. The modified technique herein described has been used on rats of all ages and has proven to be highly satisfactory in all cases and definitely superior to the classical method of clipping off the tip of the tail for blood samples.

Second, liver and certain liver fractions have been shown to aid the rat in maintaining a high level of polycythemia when the animal is fed cobalt. These results are in agreement with the observation of Marshall (2) although our data are not directly comparable since in our study the animals received the supplements at the same time that cobalt administration began while Marshall fed the supplements after the polycythemia had been produced.

Our observations are interesting in light of the effect of choline hydrochloride or liver administration which has been reported by Davis (5) to depress cobalt polycythemia in dogs. The liver extract (Lilly) failed to depress polycythemia in the rats although it was fed at a level which would supply approximately 8 mgm. of choline per kilogram of body weight, the level found to be effective for dogs. The supplemental effect of certain liver fractions for the maintenance of polycythemia indicates that choline does not have a depressing action on the cobalt polycythemia of rats at the level fed.

Since we were able to maintain the control animals at high levels of polycythemia by modifying the bleeding technique, it would appear that the level of Mn employed was adequate, although the effects of higher levels were not studied.

SUMMARY

1. Fluctuations in the hemoglobin level of polycythemic rats have been largely prevented by modifying the technique of blood sampling to prevent unnecessary losses of blood.

2. Whole liver powder, liver extract (Wilson's) and a P. A. concentrate for oral use (Lilly) were found to aid the rats in producing and maintaining a higher level of cobalt polycythemia when added to a milk diet.

3. Casein had a very slight supplementary effect while liver residue (Wilson's) gave results identical with the control group.

4. When the calcium-phosphorus ratio of the milk was decreased to 0.6 by the addition of NaH_2PO_4 the rate of development of polycythemia increased with a resulting toxicity and depression of growth.

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TYPICAL ANAPHYLAXIS IN THE DOG IN THE ABSENCE OF THE LIVER

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Anaphylaxis is characterized in certain species by the response of some particular tissues, leading to asphyxia in the guinea pig, heart failure in the rabbit and hepatic engorgement in the dog. These responses are, in general, regarded by students of anaphylaxis as the most obvious feature of a widespread tissue involvement. Even so, it has been generally accepted that anaphylaxis in the dog is not only characterized by liver involvement but does not occur in the absence of this organ. This view has been based on the experimental work of a number of investigators, who have interpreted their negative findings in this way. Also it has been supposed that any responses in extra-hepatic tissues are dependent upon the primary reaction in the liver and are the result of the liberation therefrom of toxic material into the circulation.

While there are differences of opinion on the mode of action of the liver in anaphylaxis in the dog, there has been almost complete agreement that this organ is essential for the production of typical canine anaphylaxis. Manwaring (1910) was unable to elicit anaphylactic shock in dogs with livers excluded by ligating various blood vessels. Voegtlin and Bernheim (1911) and Denecke (1914) excluded the liver by preparing Eck fistulas and clamping the hepatic arteries; subsequent injection of antigen gave no shock. Dale (1912) performed a few similar experiments and obtained no trace of shock with the liver excluded from the circulation, but on release of the clamp on the hepatic artery shock did develop. Weil (1917), demonstrating the marked congestion of the liver when antigen was injected into the |portal vein or one of its branches, concluded that the congestion was a purely local phenomenon in the dog and was the only mechanism which takes part in the production of the vasomotor shock of anaphylaxis. Manwaring *et al.* (1925) found that their sensitized dogs, eviscerated or dehepatized by a modification of the Dale-Laidlaw Eck fistula technique, did not show the typical fall in blood pressure. When they mechanically obstructed the hepatic veins of a sensitized dog, Simonds and Brandes (1929) detected no further fall in arterial blood pressure when the antigen was injected, but it did occur on release of the obstruction. Again

these experiments were interpreted as further evidence that the liver was essential for the manifestation of canine anaphylaxis.

On the contrary, Pelz and Jackson (1918) claimed to have obtained anaphylactic shock after excluding the liver, but the single blood pressure tracing which they reproduce by no means represents the typical profound fall in blood pressure of the intact shocked dog. These experiments, in common with many another where exclusion of the liver was attempted by ligation procedures, may be criticized on the grounds that this organ was not completely isolated from the circulation. Thus Rich (1923) has shown by his experiments of injecting India ink into the jugular veins of dogs with ligatures around the portal vein, hepatic artery and inferior vena cava above the diaphragm, that some connection still exists by means of certain diaphragmatic vessels between the liver and the general circulation. Clearly it is preferable completely to remove the liver from the abdomen by surgical procedure. This we have done, and have obtained in these dehepatized sensitized dogs typical anaphylactic shock on injection of antigen. A preliminary account of these experiments has already been given (Waters, Markowitz and Jaques, 1938).

EXPERIMENTAL. The dogs were sensitized to horse serum which had been treated with alum. The serum was mixed with $\frac{1}{10}$th of its volume of a 10 per cent solution of potassium alum ($Al_2(SO_4)_3K_2SO_4$ $24H_2O$). This mixture was left in the refrigerator for 24 hours, then injected subcutaneously in amount equivalent to 0.5 cc. serum per kilo body weight. The shock dose of normal horse serum was given intravenously five or more weeks later. This method of sensitization we have found very satisfactory. The time required for sensitization appears to be longer than when untreated serum is given, but the animals remain highly sensitized for a much longer period. These results are in accord with the success obtained in sensitizing guinea pigs with ragweed pollen extracts treated with alum (Caulfield, Brown and Waters, 1935).

The hepatectomy operations were carried out under ether anesthesia by the one-stage technique described by Markowitz, Yater and Burrows (1933).

Dog 1.   7.2 kgm.   Sensitization period: 8 weeks.
   10:15 a.m.   Ether anesthesia.
   11:00 a.m.   Liver removed from abdomen.
   11:10 a.m.   20 cc. 25 per cent dextrose solution.
   11:26 a.m.   20 cc. horse serum (jugular vein) injected over a period of 40 seconds.
   11:55 a.m.   Dog died.
             Blood pressure (carotid) decreased evenly from 108 to 52 mm. Hg in 100 seconds of the beginning of the injection of serum. Further gradual fall until death.
             Bladder response within 30 seconds of the beginning of serum injection.

Dog 2.   10.6 kgm.   Sensitization period: 6 weeks.
 11:30 a.m.   Ether anesthesia.
 12:15 p.m.   Liver removed from abdomen.
 12:30 p.m.   15 cc. 25 per cent dextrose solution.
 12:50 p.m.   20 cc. horse serum (jugular vein).
              15 cc. 25 per cent dextrose solution injected at ½ hour intervals.
  2:37 p.m.   Further injection of 20 cc. horse serum had no effect on the blood
              pressure (42 mm. Hg).   Blood pressure decreased from 124 to 48
              mm. Hg, sharply at first, then more gradually until 10 minutes
              after serum injection.
              Bladder tone increased within 1½ minutes.   Remained contracted
              for ¾ hr.
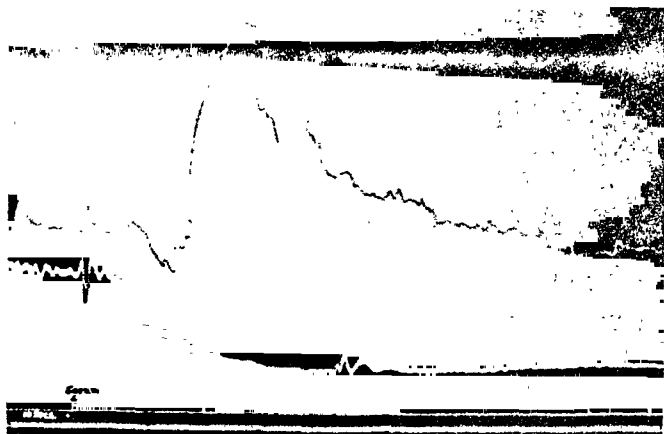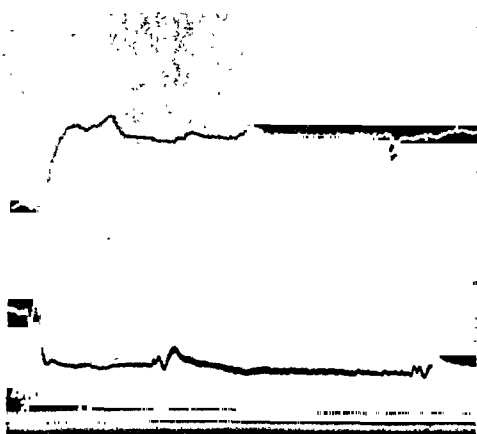
Fig. 1   Fig. 2

Fig. 1. Liverless dog 3.   Time intervals 10 seconds.   Bladder (upper tracing) carotid blood pressure (lower).   Injected with 1.3 cc. horse serum per kilo body weight.   Slight elevation of blood pressure near center of tracing due to injection of dextrose solution.

Fig. 2. Liverless dog 4.   Time intervals 10 seconds.   Bladder (upper tracing) carotid blood pressure (lower).   Injected with 0.5 cc. horse serum per kilo body weight.   Two slight elevations of blood pressure due to injections of dextrose solution.

Four sensitized dogs, after fasting for 24 hours, were dehepatized under ether anesthesia, and while still anesthetized showed anaphylactic symptoms on injection of antigen.   Details of two of these experiments are tabulated, and the kymograph records of the other two are reproduced in figures 1 and 2.   Changes in bladder tone were obtained with a tambour connected to a cannula inserted through the bladder wall.   Quantitative records of changes in pressure within the bladder were not made.   Adequate dextrose was given to prevent hypoglycemia.   The amounts of blood found in the abdominal cavity at post mortem were small.

DISCUSSION.   We have used as our criterion of shock the pronounced fall in arterial blood pressure which immediately follows the injection of the shock dose of antigen.   This was first noted by Biedl and Kraus (1909) and has been amply confirmed by all who have tested it.   We have given

some of our sera, in equivalent doses, to seven normal and to two evis-
cerated dogs under ether anesthesia, without any effect on the arterial
blood pressure except in one of the normal animals which did show a fall
in pressure of about 15 mm. Hg, but with complete recovery in about a
minute. It is therefore evident that anaphylaxis occurred in these dogs
in the complete absence of the liver, as judged by the most characteristic
test, namely, the profound fall in blood pressure.

Our results appear to support the original conclusion of Biedl and Kraus
(1909) that the decreased blood pressure is due to a primary peripheral
vasodilatation, though it would be unwarranted to conclude that in the
intact animal the hepatic changes may not be a contributing factor of some
magnitude. Indeed, our tracings indicate that the fall in blood pressure
takes place a little more gradually in the dehepatized than in the whole
animal. But some of this difference may be due to the major operation
which immediately preceded the injection of antigen. After noting the
shock that developed in otherwise normal dogs into which had been trans-
planted livers from sensitized dogs, Manwaring, Hosepian and O'Neill
(1925) considered it probable that extra hepatic factors are operative in
acute canine anaphylactic shock.

Manwaring *et al.* (1925) were of the opinion that the tonic contraction
of the bladder in canine anaphylaxis is due to the liberation from the liver
of an anaphylatoxin with a histamine-like action. We have obtained the
tonic contraction of the bladder in the absence of the liver. It has been
clearly shown by several investigators (Dragstedt and Mead, 1936; Code,
1939) that histamine is liberated in canine anaphylaxis. It is difficult to
decide whether the bladder response we obtained, in the absence of the
liver, was due to a direct action of the antigen or to the production else-
where of circulating histamine or similar substance. Dale and Laidlaw
(1910) suggested that the increase in tone of the urinary bladder of the dog
in histamine shock was due to anemia of the sacral portion of the spinal
cord. Whatever the cause of the contraction of the bladder in anaphylac-
tic shock the production of an "anaphylatoxin" by the liver is not essential,
for contraction occurs in the absence of that organ.

SUMMARY

Typical anaphylactic shock has been obtained in sensitized dogs after
complete surgical removal of the liver.

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INVOLUTION OF THE THYMUS OF THE YOUNG ALBINO RAT UNDER TREATMENT WITH TESTOSTERONE PROPIONATE

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It has been demonstrated that there is a distinct relationship between the thymus and the sex glands, in which the gonads exert án inhibitory effect. Using rats, Moore (1) showed that Antuitrin S stimulates hormone secretion by the testes, and Butcher and Persike (2) demonstrated that this increased hormone production arrests the growth of the thymus. This effect is through the gonads, since injections of Antuitrin S prove ineffective in arresting the growth of the thymi of castrated animals. On the basis of these observations, it seemed probable that testosterone propionate might induce regressive changes in the thymus, and this study was undertaken to determine the effect in young rats.[1]

METHODS. Throughout these experiments, only healthy litter-mate albino rats within 5 grams' body weight of each other were utilized, to avoid so far as possible the variations in thymic weights found in rats from different litters. No animals were autopsied at more than 100 days of age, at which time the thymus of the white rat generally begins involution (4). It has been demonstrated that the thymus reacts rapidly to disturbances in nutrition (5, 6, 7), so each group was kept in a separate cage, in which there was an excess of food and water at all times.

Each of these litter-mate groups consisted of several rats of the same sex, one serving as a control, the others being injected intraperitoneally with different amounts of testosterone propionate dissolved in sesame oil (0.1 mgm. to 1.0 cc.). There was an equal number of male and female groups. Usually, each rat was autopsied one day after the last injection. Body weights at the beginning of injections and at the time of autopsy were recorded. The relative weight of the thymus of each of the injected animals was compared to that of its litter-mate control, and the difference recorded in percent. The thymi of the majority of both experimental ani-

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[1] After these experiments were started, Chiodi (3) reported that the thymi of white rats undergo atrophy under the influence of testosterone. Since the results of my experiments differ in certain respects from his, and since the data presented here are more complete than those obtained by him, it was deemed advisable to present this work.

mals and their controls were also examined for histological changes. Livers from most of the rats were examined in sections stained with hematoxylin and eosin and in frozen sections stained with Sudan III.

As approximately 150 litters, with a total of 357 rats, were used in these experiments, not all of the data obtained can be presented here. Typical litters, therefore, have been selected, and the data from these are presented in table 1.[2]
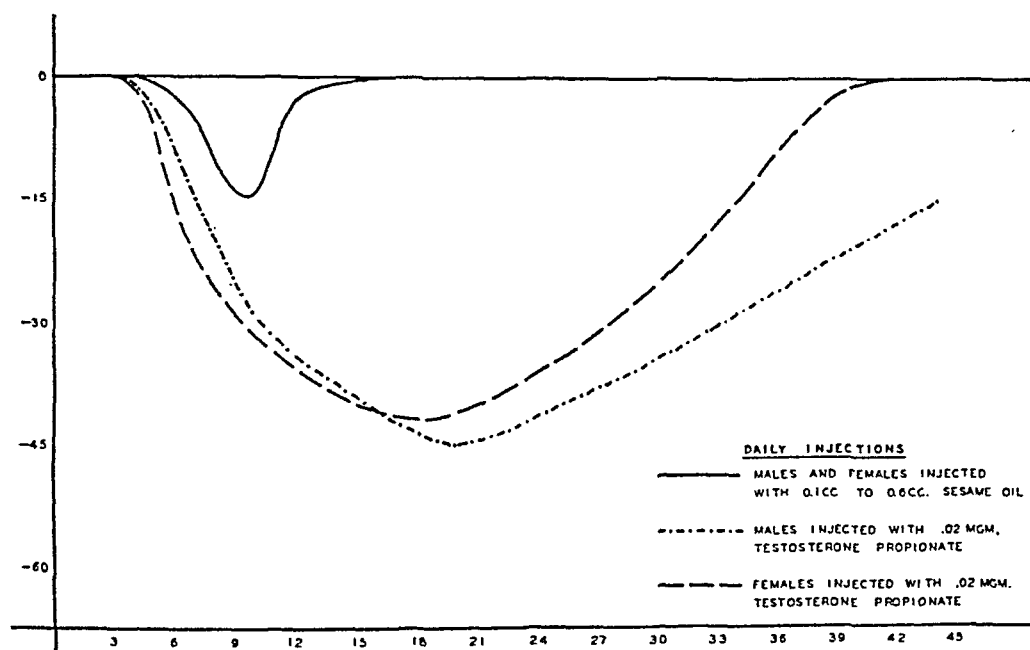
Fig. 1. The number of days of injections (x-axis) is plotted against the per cent of decrease (−) in the weight of the thymus of the injected rat as compared with the weight of the thymus of its litter-mate control (y-axis). The individual percentage points for each day of injections are averaged, and a smooth line graph is drawn from the average points thus obtained.

OBSERVATIONS. After 4 to 5 daily injections of 0.02 mgm. or more of testosterone propionate, the thymi of both male and female rats, between the ages of 20 days and 80 days, begin to involute. This involution reaches a peak at about the 19th day of injections. Figure 1 indicates that this decrease in size of the thymi of the injected animals is an involution and not an arrested growth.

The histological structure of the thymi of the injected rats changes slowly. By the 22nd day of injections, however, it is clearly evident that there are fewer thymocytes in both cortex and medulla, resulting in a less distinct differentiation between these two zones. The capsule becomes thickened,

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[2] The complete data are on file in this laboratory, and will be made available to anyone who wishes to consult them.

TABLE 1

Representative protocols

SEX	GROUP NUMBER	NUMBER OF RATS	TREATMENT	NUMBER OF DAYS OF INJECTIONS	Age	Body weight	Thymus weight	Per cent +	Per cent −
						grams	milligrams		
♀	1	1	Control		81	74	220		
		1	0.01 mgm. daily from 61st day	20	81	72	178		16.9
♂	2	1	Control		80	58	145		
		1	0.01 mgm. daily from 62nd day	18	80	52	92		31.9
♀	3	2	Controls		74	76.5	218		
		1	0.02 mgm. daily from 55th day	18	74	72	127		37.7
		1	0.03 mgm. daily from 55th day	18	74	76	136		37.6
♂	4	1	Control		52	56	124		
		1	0.02 mgm. daily from 36th day	16	52	51	64		43.3
		1	0.03 mgm. daily from 36th day	16	52	58	77		40.4
♀	5	1	Control		62	111	213		
		1	0.02 mgm. every other day from 41st day	11	62	109	177		15.3
♂	6	1	Control		45	59	117		
		1	0.02 mgm. every other day from 28th day	9	45	56	79		28.8
♂	7	2	Controls		10	14	20.5		
		1	0.02 mgm. daily from 3rd day	7	10	12	15		11.8
		1	0.02 mgm. daily from 3rd day	7	10	8	10		14.5
♀	8	1	Control		6	9	13		
		1	0.02 mgm. daily from 3rd day	3	6	6	8		8.0
♀	9	1	Control		20	25	84		
		3	0.02 mgm. daily from 13th day	7	20	26	68		22.7

TABLE 1—*Concluded*

SEX	GROUP NUMBER	NUMBER OF RATS	TREATMENT	NUMBER OF DAYS OF INJECTIONS	Age	Body weight	Thymus weight	Per cent +	Per cent −
						grams	milligrams		
♀	10	1	Control		64	73	105		
		1	0.02 mgm. daily from 27–43rd days	17	64	79	152	33.3	
♂	11	1	Control			97	157		
		1	0.02 mgm. daily from about the 30th day for 16 days. Killed 21 days after last injection	16		110	193	8.4	
♀	12	1	Control			69	149		
		1	Castrated. 5 days later, 0.02 mgm. daily from about the 35th day	20		67	154	6.2	
♂	13	1	Control		69	110	216		
		1	Castrated. 4 days later, 0.03 mgm. from 58th day	11	69	110	212	2.0	
♂	14	1	Control			95	139		
		1	Castrated. 4 days later, 0.04 mgm. daily from about the 35th day	21		88	83		35.6
♂	15	1	Control			107	220		
		1	Castrated. Killed 21 days later			101	255	25.0	
		1	Castrated. 5 days later, 0.08 mgm. daily from about the 35th day	20		87	57		68.1

and there is an invasion and replacement of the normal thymic tissue by connective and adipose tissues.

Since the hormone was administered in oil, tests were made to determine the effect of the oil itself on the growth of the thymus. Thirty groups of animals were injected daily with sesame oil, in amounts ranging from 0.1 cc. to 0.6 cc. These injections did cause the thymus to involute slightly for a short time (fig. 1), the smallest injection producing about the same effect as the largest. By the 13th to 15th day of injections, however, refractoriness to the oil developed, and the weights of the thymi of the injected animals returned to normal (fig. 1).

To determine the smallest amount of testosterone propionate which would produce a maximum involution of the thymus of the average rat used, daily injections were varied from 0.01 mgm. to 0.04 mgm. Injections of 0.01 mgm. produced a moderate degree of involution (litters 1, 2: table 1). On the basis of data obtained from 24 groups, with a total of 76 rats, it would seem, however, that 0.02 mgm. was the optimal daily dosage, since it was as effective as daily injections of 0.03 mgm. and 0.04 mgm. (litters 3, 4).

Three groups of males were injected with this most efficient dosage (0.02 mgm.) every other day for a total period of 17 days. A like number of females was similarly treated for a total period of 21 days. While a certain degree of involution was obtained in all of the injected animals of these two groups, it was found that the percentage involution in these rats was not so great as that which occurred in those animals injected daily with the same dosage. (Compare litters 3, 4 with litters 5, 6.) For producing a maximum degree of thymic involution in the average rat used, the daily administration of 0.02 mgm. of testosterone propionate appears to be most efficient.

To determine the earliest age at which a change in the thymus could be induced, 10 groups of rats were injected daily with 0.02 mgm. of testosterone propionate from the second day of life, and were killed at various ages. In these very young rats, the effect of the injections was generally so great as to inhibit the body growth to such an extent that the injected animals usually weighed about one-fourth less than their controls, after only 6 to 7 days (litter 7). In these groups it was apparent that a decrease in the size of the thymus could be obtained at the 6th day of age (litter 8). It is a question, however, how early testosterone propionate itself will cause a change in the thymus, since the reduction in the size of the thymi of the rats in these groups may have been caused, not by the hormone, but by disturbances in nutrition, as is indicated by the failure of the injected animals to grow as much as their non-injected controls. On the other hand, rats injected with 0.02 mgm. of the hormone from the 13th to 19th day of life showed a very distinct involution of the thymus, accompanied by no disturbances in body growth (litter 9).

In gonadectomized animals injected with testosterone propionate, a regression in the size of the thymus was also obtained. Daily injections of 0.02 mgm. and 0.03 mgm., however, were not sufficient to definitely cause the thymus of the gonadectomized rat to involute (litters 12, 13) but injections of 0.04 mgm., 0.06 mgm. and 0.08 mgm. produced a definite reduction (litters 14, 15). Non-injected gonadectomized rats showed either unaffected or larger thymi than did their controls.

Certain rats were injected for long periods of time in order to determine whether or not refractoriness would develop. Females, injected daily

with 0.02 mgm. of testosterone propionate, exhibited refractoriness, since their thymi returned to normal size at about the 44th day of injections (fig. 1). Males, comparably treated, showed a certain degree of refractoriness by the 44th day, although this effect was not so pronounced or complete in the males as it was in the females (fig. 1). This sex difference appears to be statistically significant, since the probability against a difference of this magnitude occurring by chance is more than 1000:1.[3]

Eight groups of rats were injected with 0.02 mgm. of testosterone propionate for 17 days, and were autopsied at about 20 days after the last injection. The thymi of these rats had hypertrophied, and, in most cases, exceeded in weight the thymi of their litter-mate controls (litters 10, 11).

At autopsy, it was found that out of 139 injected animals, 28 had grown less, 18 had grown more, and 93 had grown neither more nor less than their controls. Since almost comparable numbers of the injected rats were either heavier or lighter than their controls, and since the great majority of the animals were unaffected at all by the injections, it would appear that daily administration of 0.02 mgm. of testosterone propionate was unable to bring about an effect on the growth or body weights of the injected animals, after the first 12 days of life.

Nevertheless, in spite of this lack of influence on growth rate, frozen sections stained with Sudan III., and fixed sections stained with hematoxylin and eosin from livers of rats injected with either sesame oil or testosterone propionate dissolved in oil showed marked fatty changes of the organs. In some cases, fatty infiltration occurred, particularly on the periphery of the livers, where they were in direct contact with the oil injected into the abdominal cavity. There was also some evidence of a mild chronic inflammatory reaction, probably as a result of the subjection of the hepatic tissue to a foreign substance.

The testes of the male rats, and the ovaries of the female rats which were injected with the hormone were smaller than those of their litter-mate controls. This inhibitory effect upon the gonads occurred gradually, but in the rats injected for longer periods of time, it was quite marked. The kidneys of rats injected with 0.02 mgm. of testosterone propionate generally were larger than those of their controls. This increase varied from 8 per cent to 25 per cent.

DISCUSSION. The histological changes in the thymi which had undergone induced involution are similar to those occurring in normal involution, as described by Maximow and Bloom (8), and are much the same as those found by Butcher and Persike (2), Chiodi (3) and Smith (9).

The finding based on data obtained from many groups of rats that injections of sesame oil do have a depressive effect on the thymus for a short

[3] I am indebted to Prof. F. W. Weymouth for suggesting the statistical analysis and for checking the results.

time is not in accord with the report of Chiodi (3), who states that the decrease in the size of the thymus cannot be attributed to the trauma or toxicity of the oil vehicle in which the hormone was dissolved. As his animals were injected for 11 days with 0.5 mgm. of testosterone dissolved in 0.33 cc. of the same oil, and were killed 4 days after the last injection, I believe that some of the thymic involution obtained by him might have been directly due to the adverse effects of the oil in which the hormone was dissolved.

Chiodi (3) used two strengths of testosterone, 0.5 mgm. and 1.5 mgm., and thought that the degree of regression in the size of the thymus is related to the strength of the dosage. It would seem, though, that this inference is hardly justified, since many of our animals which were injected with only 0.02 mgm. of testosterone propionate daily showed as great a degree of thymic involution as did the relatively few used by Chiodi, which received so much more of the hormone for the same period of time—unless one is to infer that the difference in potency of the two preparations is in the ratio of about 75:1.

It is of more than passing interest that the thymi of those rats injected for 17 days and killed 20 days after the last injection hypertrophied rapidly after the injections were stopped. Butcher and Persike (2) also found this to be true in rats comparably treated with Antuitrin S. These results are in accord with those of Gottesman and Jaffe (10), who state that thymic remnants undergo hypertrophy after removal of the greater portion of the thymus. This suggests that there is some factor which maintains the thymus during immaturity. Chiodi (3) also found that the thymi of gonadectomized rats can be made to involute if the animals are treated with male sex hormone. That he failed to notice the difference in threshold is probably due to the fact that all of his dosages were excessive. Injections of testosterone propionate and the attendant organ changes had no appreciable effect on the body growth of the injected animals. Chiodi (3) and McEuen, Selye and Collip (11) also found no changes in body growth as the result of male hormone administration. Although this was generally true in the present series, very young animals were inhibited in their growth, as has been discussed above. Selye (12), using mice, likewise found that the kidneys of those animals receiving testosterone propionate were usually enlarged.

SUMMARY

The thymi of young albino rats of both sexes under 100 days of age were made to involute by injections of testosterone propionate, this involution reaching a peak at about the 19th day of injection. That this effect is significant is indicated by statistical analysis, which shows that the chances of such deviations as those observed being fortuitous are less than

1 in 1000.   Daily administration of 0.02 mgm. or more caused the maximum degree of thymic involution in both sexes.   This change, starting at about the 4th to 5th day of injection, was a real involution, not merely an arrest in growth.   Refractoriness to prolonged injections of testosterone propionate occurred in both males and females, being more pronounced in females than in males.   When the daily dosage was increased to 0.04 mgm. or more of testosterone propionate, an involution occurred in the thymi of gonadectomized animals of either sex.   The thymi of both male and female rats hypertrophied when the injections were stopped.   Injections of pure sesame oil caused a slight degree of thymic involution for a short time, after which refractoriness occurred.   Testosterone propionate administration did not affect the body growth of the injected rats of either sex, except at early ages.

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ACTION POTENTIAL ARTIFACTS FROM SINGLE NERVE FIBERS

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The external, measurable action potential from a single element, the nerve fiber, has often been compared (cf. Gasser and Erlanger, 1927) to the potential drop across an external resistance R_e between the poles of a battery with internal resistance R_i. If the battery has an e.m.f., e, then

$$e = i(R_e + R_i), \tag{1}$$

where i is the current. Therefore in this analogy the external potential drop, the spike height, iR_e is determined by e, R_e, and R_i:

$$iR_e = e \frac{R_e}{R_e + R_i}.$$

e is the potential difference across the polarized membrane, R_e the longitudinal resistance outside the fiber, measured from the point where the crest of the impulse finds itself to the foot or the end of the spike, and R_i the corresponding resistance of the core of the fiber plus the resistance of the membrane at the electrically permeable region.

It is therefore to be expected that the size of the action potential can be modified by agents (fluids, metals, etc.) which change R_e. Any sudden change in the electrical conductance outside the fiber should abruptly change the action potential as it passes and theoretically should produce irregularities or artifacts in the recorded potential.

The purpose of the present paper is to investigate the effect of varying the electrical conductance external to single, isolated, unmyelinated fibers upon their electrical response and the potential field surrounding them during activity. The "artifacts" produced as a consequence of irregularities in conductance between the leads will be studied, and several of the more interesting ones will be analyzed.

A method of leading off with the fiber surrounded by a small quantity of solution was developed for these experiments. This method commends itself in general because there is little chance of damage to the fiber. It

is applicable to the study of other problems involving single fibers, particularly the action of pharmacological agents.

METHOD. Single nerve fibers were obtained mainly from the claw of the crayfish, Cambarus clarkii; they were prepared according to the method developed by van Harreveld and Wiersma (1936). For the most part the large motor axons of 20 to 40 μ diameter were isolated since these can be readily obtained free from connective tissue, which is not true of the smaller sensory fibers.

The prepared fibers, isolated for a distance of 20 to 25 mm., were either dissected from the claw with a bundle of nerve left attached at either end for ease in clamping, or else the proximal end was prepared in this way and the distal end left attached to the claw, the joints from which the fiber was prepared being discarded. The fiber was transferred to the leading off chamber, which had been filled with van Harreveld's (1936) solution, and the end, or ends, secured. If the fiber was left attached to the claw, the latter was held outside the end of the chamber, the nerve entering through a slot in such a way that it was kept covered with solution. Keeping the claw and therefore the muscle which the axon innervated attached to the preparation was done because the fiber maintained its activity much longer than one severed completely: 6 to 8 hours or more against one hour or less.

The leading off chamber employed in the experiments consisted of a triangular trough cut into a strip of Lucite which formed the bottom of a rectangular box of the same material; see figure 1, A and B. The trough had a vertex angle of 90°. Fluid leads entered at the vertex through fine pores of about 90μ diameter, spaced at intervals of 5 mm. in the case of the leading off electrodes. The other openings of the leads were through holes drilled into the side of the box and strip. Into these were inserted, ordinarily, platinum wires connected to the stimulator and amplifier. If the wires to the latter were of silver-silver chloride, nonpolarizable electrodes were obtained which were not poisonous to the preparation because of the length of fluid lead intervening. The free ends of the preparation were held in the vertex of the trough by overhead clamps, and one of these was movable by means of a lead screw.

The amount of fluid surrounding the fiber in the chamber could be limited by "lids" for the trough; see figure 1, C. These lids were made of a square rod of Lucite, beveled along one edge. This edge fitted into the vertex of the trough so that there a triangular cross-section of definite area was obtained.

After the fiber was in place along the bottom of the trough's V and the lid to be used was in place over the axon, the excess fluid was pipetted from the chamber, care being taken to blot up any fluid that could serve as a shunt around the leads. In this way the fiber was left surrounded

during the experiment by a limited quantity of fluid which formed the only appreciable conducting medium between the electrodes.
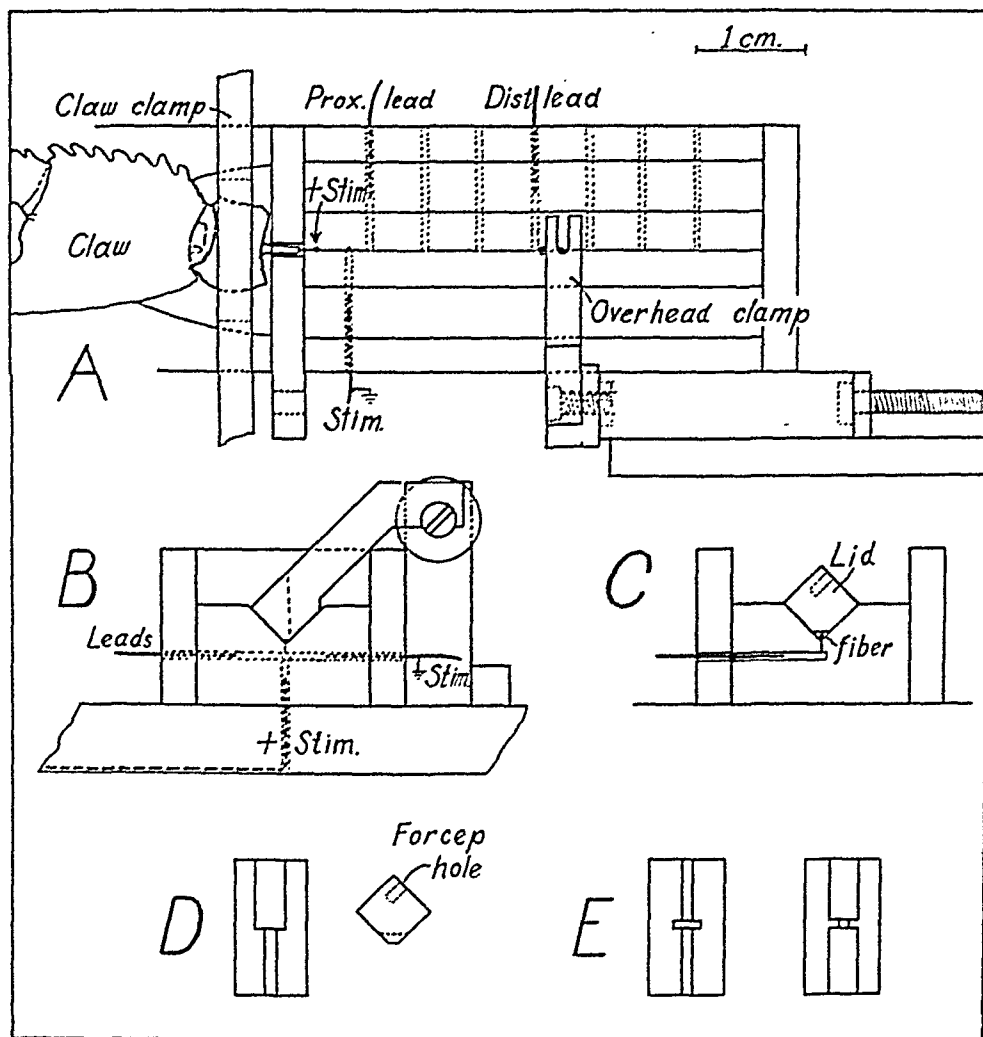
Fig. 1. Leading off chamber and lids.

A. Top view of chamber, showing trough, position of leads and preparation.

B. Side view of chamber from the stimulating, or proximal, end.

C. Cross-section of trough through a lead, with a lid and fiber in place.

D. Bevel view of lid used for producing a sudden change in conductance, and side view of this lid.

E. Bevel views of lids used for producing a sudden short increase and decrease in conductance, respectively.

The stimulator employed generated square waves whose frequency, duration and amplitude could be independently varied. The action potentials were led off through a push-pull differential amplifier into a cathode ray oscillograph. Very low grid-to-filament capacity tubes were

used in the first stages of amplification so that the current flow from the leads to ground and back through the preparation caused by the action potential would amount to less than 10^{-10} amps. Usually the stimulator and the sweep of the oscillograph were synchronized at a frequency of 30 per sec. This frequency did not produce signs of fatigue in the fiber, even after continuous stimulation for some minutes.

RESULTS. Before proceeding to a description of the artifacts produced by non-uniformity of conductance in the region between the leads, it will be necessary to consider the effect of the external conductance upon the action potential.
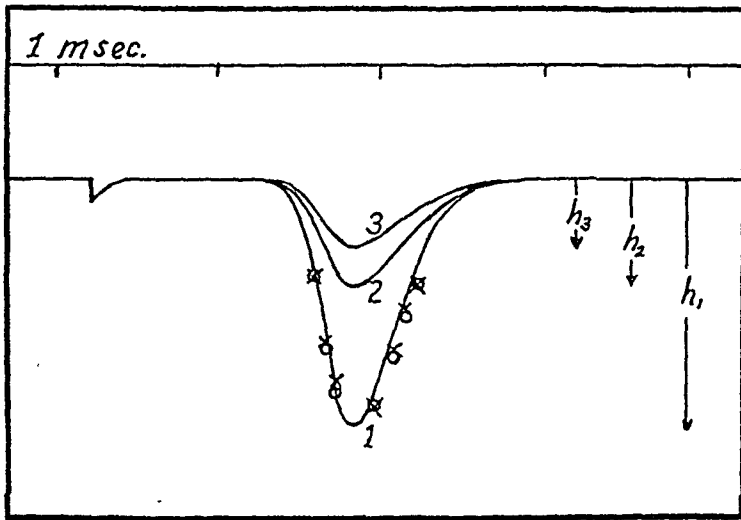
Fig. 2. Curves *1, 2* and *3* are tracings of action potentials led off from the same fiber at the same distance (7 mm.) from the stimulating electrode; only the cross-section of the trough, and therefore the conductance, was changed. The crosses are the points obtained by multiplying ordinates of curve *2* by the ratio h_1/h_2 of the spike heights of curves *1* and *2*. Similarly, the circles are the point obtained by multiplying ordinates of curve *3* by h_1/h_3.

The maximum height of the action potential, with the smallest practicable amount of fluid, was about 1.5 mv. as compared to the 20 to 50 mv. which were obtained from these fibers when mounted in air or oil (cf. Hodgkin, 1938). As the amount of fluid in the trough was increased the height of the recorded spike declined. This change in height was the only one; the shape, duration and velocity of the action potential did not differ appreciably for different amounts of fluid when each amount was made uniform in cross-section by using a lid of constant bevel width. See figure 2 for a comparison of action potentials from the same fiber. That there was no difference in velocity such as Hodgkin (1939) found for single crab fibers immersed in sea water versus oil is explained by the fact that in the present case the external resistance, which is varied, is

small compared to the internal, and therefore in equation (1) the current i, which influences the velocity, will remain almost constant.

The question arises as to how much of the actual potential drop developed externally to the fiber and parallel to its axis is picked up by the electrodes under the different circumstances. To determine this point micro-electrodes were prepared, consisting of small glass tubes with one end drawn out into a capillary having a terminal inside diameter of approximately 10 μ. One of these electrodes was connected to one of the amplifier leads by means of a silver chloride-plated silver wire which dipped into the fluid contained in the tube. The other lead was by way of the trough in the usual manner. The capillary electrode was held by a micro-manipulator so that the pore of the electrode could be placed at any position relative to the fiber.

It was found that whether the pore rested on the surface of the fiber or was removed as far away as the fluid in the trough allowed (a range of 0.1 mm. in the case of least fluid up to 1 mm. or more with larger amounts) the height as well as the shape and duration of the action potential remained the same. If more solution was added the action potential decreased, but the decrease was the same irrespective of where the electrode was placed.

The result of this experiment implies that the equipotential lines describing the potential field external to the active region of the fiber extend out practically perpendicularly from the fiber's surface, and therefore the actual potential difference developed along the axon's external surface is led off. In other words, the medium about the nerve fiber acts as a linear conductor for the action *current*.

Artifacts produced by change in conductance. Bishop, Erlanger and Gasser (1926) discovered that distortions of the action potential record from myelinated nerve arose when idle leads, drops of water, undissected tissue, dead fibers, or cut branches occurred in the led-off region of the preparation. These were described as furnishing extra leads, as indeed they may. Schaefer and Schmitz (1934) showed that some of these types of artifacts were produced in myelinated nerve by variations in the external conductance along the nerve.

Early in the present work it was discovered that small variations in the medium surrounding the isolated fiber as well as damage to it gave rise to records riddled with irregularities. One type of injury common in unmyelinated fibers unless carefully handled is constriction which would not only abruptly change the external but also the internal resistance of the fiber at the narrowed place. These were the main reasons why the trough method was developed, for with this technique the fiber could be maintained in good condition and, if the trough and lid were made precisely enough, unwanted artifacts could be avoided.

Any variation in R_e (or R_i) as the action potential is conducted between the leading off electrodes will produce a spurious response in the record. This is caused by a change in the form of the action potential which will alter the potential of one lead relative to that of the other while the change is taking place (see diagram and analysis below). These artifacts can be especially well shown in single unmyelinated fiber preparations because the shortness of the wave-length of the impulse, about 5 mm., allows the artifact to be recorded distinct from the response at the first lead.

There are several types of spurious response, which can be produced under controlled conditions, that are of special interest: 1, those caused by a sudden increase or decrease in conductance, and 2, those produced by a sudden increase or decrease limited to a short distance ($\frac{1}{2}$ to 1 mm.) of the led-off stretch of fiber. The desired variations in conductance were accomplished by regulating the cross-section of solution in the trough with the aid of the special lids illustrated in figure 1, D and E. It will be shown below that the first type of artifact is proportional to the action potential itself, and that the second is proportional to the first differential of the action potential. Figure 3 illustrates examples of the two types.

In figure 4 A the situation in case (1) is shown diagrammatically. As the impulse travels from region 1, where the conductance per unit length measured along the fiber as C_1, to region 2, where the corresponding conductance decreases to C_2, the current flow outside of and along the fiber remains practically the same but the potential drop in region 2 is greater than it would have been in region 1 because of the decrease in conductance. Therefore lead G_2 will be at a higher potential than G_1. In figure 4 B the potential along the fiber is plotted against its length; the equipotential lines in A were drawn from the potential plot in B.

It is possible to derive a mathematical expression for the resulting recorded artifact. Let

$$E_1 = E_1(x - vt)$$

be the function of the parameters x and t, the distance along the fiber and the time respectively, which will represent the traveling action potential wave in a medium having the conductance, C_1 of region 1 of figure 4; v is the velocity of the wave. Let E_2 be a similar function for the wave in a medium having the conductance, C_2, of region 2. Now if h_1 and h_2 represent the spike height in regions 1 and 2, respectively, then, at least to a good approximation,

$$E_2 = \frac{h_2}{h_1} E_1(x - vt). \tag{2}$$

This follows from the experimental fact that the shape, duration and velocity of the wave does not change under the present conditions (cf. fig. 2).
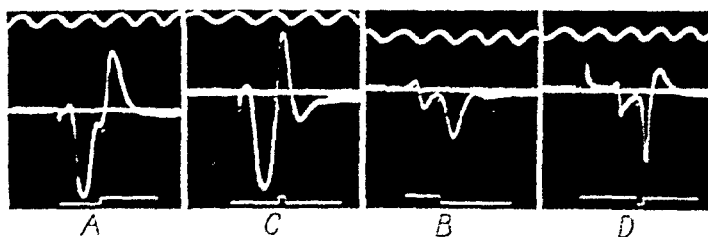
Fig. 3. Records of artifacts. In each case the change in conductance producing the artifact occurred at 6 mm. from the first lead. Therefore the first negative deflection is a record of the action potential itself, while the ensuing variations, the start of which is marked by an X, is the artifact. Under each record the change in conductance is shown diagrammatically. Monophasic leading off; time, 1 msec. See text for further references to records.
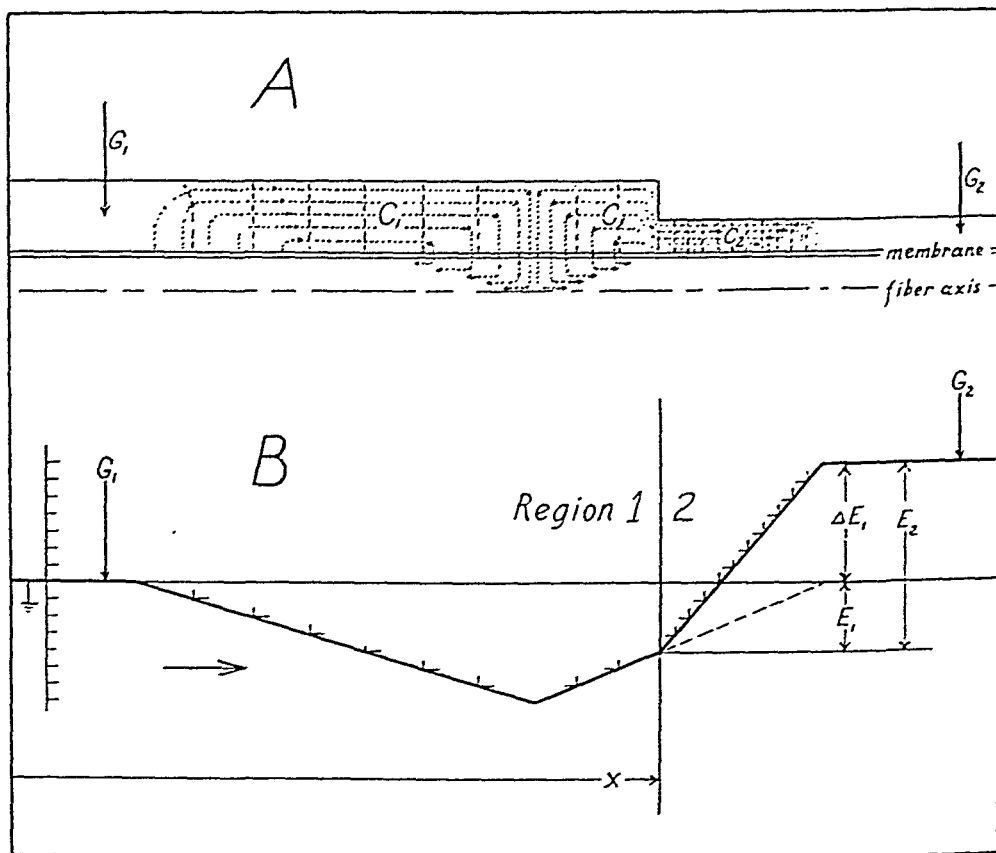
Fig. 4. A. Scheme of the current flow outside the fiber when the impulse encounters a sudden decrease in conductance. The equipotential lines are constructed from B, a diagram of the potential along the fiber. See text for analysis.

As a consequence of this fact it can be assumed that the current leaving or entering the membrane along the wave does not change as the external resistance is altered, and therefore from equation (2)

$$\frac{h_2}{h_1} = \frac{R_{e_2}}{R_{e_1}} = \frac{C_1}{C_2}$$

so that

$$E_2 = \frac{C_1}{C_2} E_1 . \tag{3}$$

Furthermore, since the external medium serves as a linear conductor, in the neighborhood of the discontinuity the part of the wave in region 1 will be represented by E_1 and the part in region 2 by E_2. Therefore

$$\Delta E_1 = E_2 - E_1, \text{ at } x,$$

where ΔE_1 is the increment of E_1 produced by the sudden change in conductance, as shown in figure 4 B. From equation (3), then,

$$\Delta E_1 = \frac{C_1 - C_2}{C_2} E_1 , \text{ at } x,$$

$$= G_1 - G_2 ,$$

where G_1 is the potential of the first lead relative to ground and G_2 that of the second. This means, of course, that the recorded artifact, expressed by the increment ΔE_2, is proportional to the action potential.

This artifact is of some use in certain types of experiment, for instance those with drugs, since effectively an extra lead can be obtained at any point in the led-off stretch by merely shifting the special lid. Examples of this type of artifact were shown in figure 3, A and B. Actually, the conditions diagrammed in figure 4 are those under which figure 3 B was taken; 3 A is the converse case.

Expressions for the artifact shown in C and D of the latter figure can also be readily derived. Referring to figure 5 A, the action potential in region 1 suddenly encounters a narrow region 2 of different conductance. Using the same notation, it will be seen that

$$\Delta E_1 = \delta E_2 - \delta E_1 .$$

Since

$$\delta E_2 = \frac{C_1}{C_2} \delta E_1 ,$$

$$\Delta E_1 = \frac{C_1 - C_2}{C_2} \delta E_1 .$$

If Δx is sufficiently small compared to the wavelength, then

$$\delta E_1 \doteq \frac{\partial E_1}{\partial x} \Delta x, \text{ at } x.$$

Therefore,

$$G_1 - G_2 = \Delta E_1 \doteq \frac{C_1 - C_2}{C_2} \frac{\partial E_1}{\partial x} \Delta x, \text{ at } x. \tag{4}$$

The recorded artifact is thus proportional to the first differential, $\dfrac{\partial E_1}{\partial x}$, of the action potential, which changes sign at the crest of the spike, accounting for the diphasic response seen in figure 3, C and D.

A spurious response very similar to the latter artifact will be recorded from a preparation where there is an abrupt but short increase in conductance, such as could be caused by an idle electrode. Of more interest,
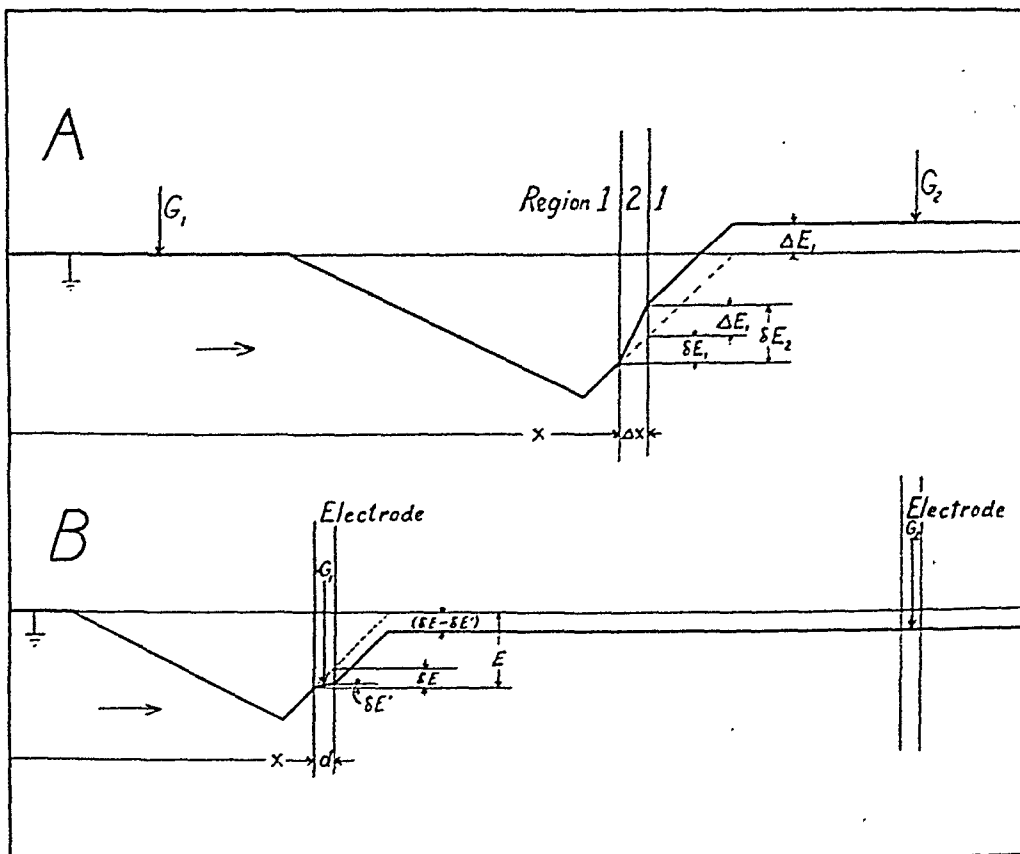
Fig. 5. A. Diagram of the potential along the fiber at an instant when the impulse is passing a sudden short decrease in conductance, the circumstance producing the artifact shown in figure 3 D. B. Similar potential diagram for the case where the impulse is passing an electrode of high conductance. See text for analyses.

however, is the question of the effect of the increased conductance furnished by the active leads. The last result, equation (4), will be of help in finding an answer.

In figure 5 B the impulse is traversing an active electrode covering the region x to $(x + d)$. If E is the function expressing the potential of the traveling wave in the region before and after the electrode, having the conductance C, and E' the function in a region having the conductance

C' of the electrode, then the potential, G_1, of the first electrode relative to ground can be expressed by

$$G_1 = E - \frac{\delta E'}{2}, \text{ at } x;$$

and the potential, G_2, of the second electrode by

$$G_2 = \delta E - \delta E' \stackrel{a}{=} \frac{C - C'}{C'} \frac{\partial E}{\partial x} d, \text{ at } x,$$

from equation (4), when d is small. When the electrode has a very high conductance so that $\frac{C - C'}{C'} \stackrel{a}{=} - 1$, the difference in potential of the electrodes can be expressed by

$$G_1 - G_2 \stackrel{a}{=} E + \frac{\partial E}{\partial x} d, \text{ at } x,$$

and from Taylor's series

$$G_1 - G_2 \stackrel{a}{=} E (x + d - vt), \text{ at } x.$$

Therefore when the first electrode has low resistance it will not start to lead off the action potential until the impulse has traversed most of the width of the electrode. This conclusion agrees with experimental evidence obtained by Bishop, Erlanger and Gasser (1926) and others.

It can be concluded that great care must be exerted when leading off from single isolated fibers, where the environment of the fiber can be easily influenced, to avoid the presence of unwanted conductance artifacts. On the other hand, here such artifacts can be most readily recognized. This is not always the case in more complicated tissue such as whole nerve or the central nervous system. It is possible that some potential variations found in these tissues and ascribed to other causes are really due to irregularities in conductance.

SUMMARY

A new method for leading off the action potentials from single unmyelinated nerve fibers is described; the fiber is mounted in a Lucite trough and surrounded by a small amount of solution.

It is found under these conditions that though the external action potential decreases as the conductance about the fiber is increased, the shape, duration and velocity of the action potential remain unchanged within the limits of experimental error. It is shown that the medium about the action potential acts as a linear conductor for the action current.

Artifacts resulting from irregularities in the conductance outside the fiber are described. Two special types of artifacts, one giving a response proportional to the action potential and one a response proportional to its first differential, are analyzed.

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SOME FACTORS WHICH INFLUENCE THE INHIBITION OF WATER LOSS IN ALBINO RATS BY PITUITRIN[1]

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Most experiments concerned with the effect of posterior pituitary extracts on body water have been performed upon mammals or frogs. In mammals the almost universal method of measurement of such effects has been the recording of changes in the volume of urine. In frogs, the usual reaction investigated is one which has been referred to as the Brunn reaction after Fritz Brunn who discovered the reaction in 1921 at Prague (3) and in this reaction an increase in total body water following the injection of posthypophyseal extracts is measured by changes in body weight. Superficially, the Brunn reaction and the mammalian "diuretic-antidiuretic" reaction appear to have little in common but a number of recent investigations in this laboratory (for example, 2) have brought to light evidence which indicates that in at least a number of respects they are fundamentally alike; apparent differences were found due to differences in the rate and nature of water exchange in the two classes of vertebrates.

In most previous investigations from this laboratory, the problem of phyletic variations between amphibians and mammals has been approached from the amphibian end. In the present study, we decided to approach it from the mammalian end. It seemed possible that the apparent differences between the Brunn and mammalian reactions might be due to differences in the method of measuring the effect of posterior pituitary extracts. Hence we decided to measure any effects not as changes in urine volume but as changes in the body weight of mammals. Using albino rats given water by mouth in the familiar water diuresis technique, we found that suitable doses of pituitrin would inhibit the loss of weight in such animals. To establish conditions necessary for the standardization of such a reaction, our first problem was to find the effect of various factors which might conceivably affect the results. We wish now to report on such factors as we have investigated to date.

Selected, mature, healthy albino rats were housed in rooms with partial

temperature control and fed on Purina Fox Chow Checkers with iodized water to drink ad libitum, except for 15–18 hours previous to an experiment when no food or water were allowed The general technique consisted in choosing some 15 to 20 rats from this colony, weighing them to the nearest 0.1 gram on a trip scale balance, placing them in individual, small cages, administering 5 per cent of their body weight of distilled water by mouth, injecting half of the number with pituitrin and subsequently re-weighing all animals every half-hour or hour. Mean percentage changes in weight were plotted against time.

Significance of control curves. The number of animals which may comprise a group is of necessity limited in any one man-day experiment. In Burn's method of assay of pituitary antidiuretic activity, each dose group consists of 4 rats (5). We have used a minimum of 6 rats to a group and the problem arose as to what variation might be expected between control groups of this number of rats. In other words, how far away should any 6-rat mean curve be from its control 6-rat curve in order that the difference be unlikely due simply to variation in controls? To get an estimate of expected variation in 6-rat control groups, 23 such groups were given 5 per cent of their weight of water by mouth and individually weighed at half-hourly intervals. The mean percentage change in weight of each group at each half-hour was calculated and a grand mean and its standard of all group means at each interval were found. The grand means have been plotted in figure 1 as a heavy solid line and the standard deviations about these grand means have been indicated as a shaded area.

The shaded area includes two-thirds of all expected means in 6-rat control groups measured under these conditions. One curve out of every six may be expected to lie above and one curve below the shaded area. Two curves, one below and one above the shaded area, would not be expected to occur more frequently than 1 out of 36 trials. We took as a working principle that when any curve lay a distance from its control which corresponded to greater than the shaded area in figure 1, a possible significant difference existed and further experiments should be done to increase the number of animals per group. In general, when the space between two curves was less than that corresponding to the extremes of the shaded area in figure 1, such curves were concluded to be insignificantly different and the factor being studied to have little or no effect, not necessarily no effect but an effect, if any, which could probably be disregarded at present in the standardization of technique.

Body weight. Heavy rats were found to lose weight, with or without pituitrin, less rapidly than small rats. This difference in control rats receiving 5 per cent of their weight of water by mouth but no pituitrin is demonstrated in the experiments summarized in table 1. Group A consisted of a total of 36 large rats and group B of 48 small rats and it is ob-

vious that during the first 2 or 3 hours, the small rats lost water at a significantly faster rate than the larger rats. After 3 hours, differences in the rates of water loss became less striking but the differences in the first 3 hours are important because it is over this interval that pituitrin is usually most effective.

In two other groups of rats similar in weight differences to those recorded above, pituitrin was injected in doses of 0.1 international unit per 100 grams' body weight. Over a 5 hour period, pituitrin retained in heavy rats 36 per cent on the average of water lost by similar heavy con-
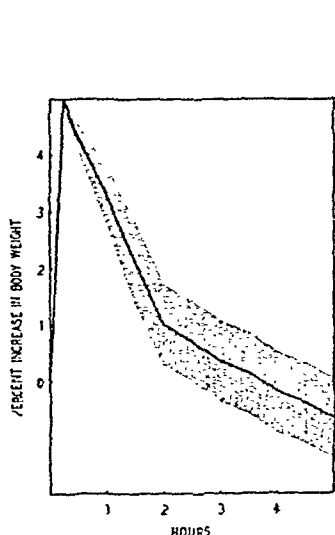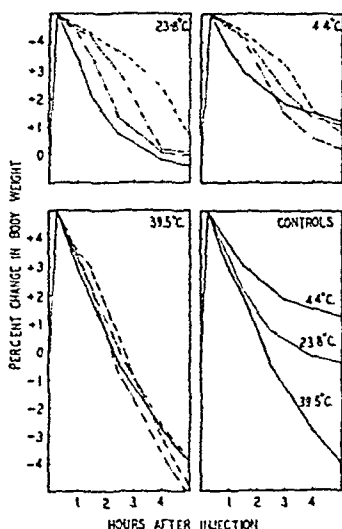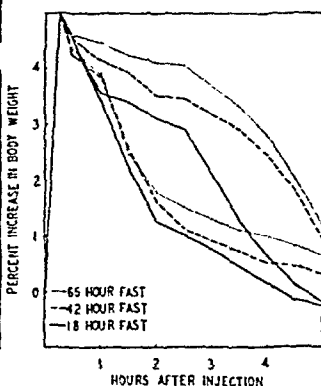
Fig. 1. Fig. 2. Fig. 3.

Fig. 1. Mean changes (solid line) in body weight of 23 groups of 6 albino rats each given by mouth 5 per cent of their weight of water and the standard deviation of the means (shaded area).

Fig. 2. The effect of environmental temperature on changes in the body weight of albino rats given water by mouth without pituitrin (solid lines) and with 0.1 international unit (single dot lines), 0.01 unit (double dot lines) and 0.001 unit (triple dot lines) of pituitrin per 100 grams body weight.

Fig. 3. The effect of various periods of fasting and thirsting on the loss of water given by mouth to rats with (top curves) and without (bottom curves) 1 international unit of pituitrin per 100 grams body weight.

trols not receiving pituitrin. Of the lightweight rats, those injected with pituitrin lost only 27 per cent less water than the controls not receiving pituitrin. The mean difference of 9 per cent had a standard deviation of 4.6 which is just about indicative of a significant difference. In subsequent experiments rats of similar weight were used in all groups to be compared.

Season. The Brunn reaction in frogs varies markedly with season, showing a markedly decreased response in the winter months in this locality (4) and in other respects we have found the body water of frogs to

respond to posterior pituitary extracts differently with seasonal changes. We anticipated a seasonal variation in albino rats but found none. When we averaged percentage changes in body weight of diuretic rats in groups of 30 or more the year around, the mean values were almost identical irrespective of season. Having excluded other suggested causes, seasonal variation in the Brunn reaction was ascribed to some internal factor such as seasonal variation in sexual activity (4). This idea is perhaps born out by the present results since we have not noted in this laboratory any significant seasonal variation in the sexual activity of albino rats as indicated by the ease and success of breeding. We have found rats a little more difficult to breed in January and February but not markedly so.

Environmental temperature. To accentuate any possible effect of environmental temperature, rats were divided into three groups and simultaneously given water by mouth with and without various doses of pituitrin

TABLE 1

The effect of body weight on the loss of water administered by mouth in doses of 5 per cent of body weight to albino rats

VALUE	GROUP	BODY WEIGHT	HOURLY PER CENT CHANGE IN BODY WEIGHT				
			1 Hr.	2 Hrs.	3 Hrs.	4 Hrs.	5 Hrs.
		grams					
Mean..................	A	250	+3.85	+2.18	+1.07	+0.23	−0.35
Mean..................	B	165	+3.34	+1.12	+0.54	+0.02	−0.49
Mean difference..........		85	0.51	1.06	0.53	0.21	0.14
Standard deviation of mean difference........		18	0.24	0.26	0.35	0.38	0.35

in one room at a temperature of 4.4°C., in another at 23.8°C., and in a third at 39.5°C. Mean changes in several experiments are shown in figure 2. At room temperature of 23.8°C., the usual type of response was obtained. In a refrigerator room at 4.4°C., rats not receiving pituitrin lost water much less rapidly, the various doses of pituitrin had a less marked and more brief water-retaining effect and in many instances the pituitrin-injected rats had actually lost more water at the end of the experiment than had the controls. At a temperature of 39.5°C., the loss of water was so rapid that pituitrin was ineffective in influencing the changes in body weight. These results indicated that environmental temperature was a factor which required itself to be taken into consideration. In subsequent experiments requiring comparison of data obtained on several different days especially in the summer months when environmental temperature varies here a good deal more than in the winter months in artificially heated

rooms, the factor of temperature was taken into account by including in each day's experiment equal numbers of rats in all groups to be subsequently compared.

Light. Environmental light was investigated because of the well known controversy regarding its reputed effect on the hypophysis and its hormones. Rats were assembled, one group in a dark room in which weighings were made with the aid of a small red light, and another group in the open laboratory. Under these conditions, no appreciable differences were noted in the weight loss of rats with or without injections of pituitrin in various doses.

Air velocity. Rats were assembled in three different rooms in which the temperature and relative humidity were the same but air velocity varied by the use of electric fans. The cooling power of the air in each room was measured by a katathermometer and the dry kata or DK readings were respectively 2.1, 3.1 and 7.9 millicalories per square centimeter of instrument per second. Weight changes in rats assembled in these three rooms with or without pituitrin were identical in corresponding experiments. Weight changes in frogs assembled under similar conditions are markedly affected by air velocity. The difference between frogs and rats is no doubt due to the fact that frogs normally lose a great deal of their water by evaporation through the skin while rats lose very little water in this manner at ordinary temperatures.

Relative humidity. No direct data were obtained on experimental alteration of relative humidity alone but in this connection the following information may be of interest. In the summer, relative humidity readings taken in the laboratory were high, between 50 and 80 per cent saturation, while in the winter with a hot water heating system the relative humidity readings fell to between 10 and 30 per cent saturation. When the results in comparable experiments with animals of the same weight, at the same room temperature, etc., were tabulated and plotted, it was not possible to demonstrate any difference. In this comparison, however, two factors were varying, season and relative humidity and it is just possible that there may have been changes in one which counterbalanced changes due to the other.

Fasting and thirsting. Rats were divided into three groups, one group deprived of food and water for 18 hours, another for 42 hours and a third for 66 hours. Rats in each group were then given water by mouth and varying doses of pituitrin. Mean changes in body weight under these conditions have been plotted in figure 3; the dosage of pituitrin used in the experiments plotted in figure 3 was 1 international unit per 100 grams' body weight. Other doses of pituitrin were also used and the resulting curves were distributed similarly to those shown in figure 3.

The results demonstrated first that a water diuresis could be produced in rats even after several days of thirsting and fasting, a finding which has been previously recorded by Heller and Smirk (6). Pituitrin was more effective in retaining body water in the more dehydrated and fasted rats and this was seen in all experiments. This is an interesting finding since it suggests that the action of pituitrin in rats can be altered by the state of water balance of tissue cells, a conclusion also reached by a different method in experiments reported by Pickford (9).

Salt intake. In the experiment just described, two factors were altered, the water intake and the food intake and probably, though not necessarily, the important part of the food intake was its salt content. Baldes and Smirk (1) recorded that a salt-deficient diet decreases water diuresis and this was confirmed by McCance and Widdowson (7). Unna and Walterskircher (10) demonstrated that pituitrin is markedly diuretic if animals are placed upon a high salt diet. In confirmation of these and other reports, we have found that giving from 50 to 250 mgm. of sodium chloride per 100 grams per day also causes pituitrin to stimulate weight loss instead of retention in rats given water by mouth. These and similar observations are all probably closely related to the original finding of Motzfeldt (8) that pituitrin cannot inhibit a saline diuresis. The phenomenon presumably occurs when the water normally retained by pituitrin is required to dissolve excessive amounts of salt, the urinary excretion of which is normally stimulated by pituitrin.

Chlorbutol in pituitrin. Commercial ampules of pituitrin contain 0.5 per cent of chlorbutol. We have found that chlorbutol itself in doses up to 1 ml. of 0.5 per cent per 100 grams body weight, which corresponds to the chlorbutol in 20 units of Pituitrin Surgical, has no appreciable effect on the loss of water administered to rats.

SUMMARY

When water is administered by mouth to albino rats and loss of body water measured as changes in body weight with and without pituitrin, the following factors affect the reaction. Small rats lose water more rapidly than large rats and pituitrin is less effective in preventing or inhibiting weight loss. Extremes of environmental temperature, either cold or hot, depress the water-retaining effect of pituitrin. Pituitrin retains water more effectively in rats dehydrated and thirsted for a prolonged period. Administered salt decreases the water-retaining effect of pituitrin and may reverse it. Factors which were found to have little or no effect on the reaction of albino rats to pituitrin included season, environmental light, air velocity and relative humdidity.

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THE BALANCE OF PHYSICAL FORCES WHICH DETERMINE THE RATE AND DIRECTION OF FLOW OF FLUID THROUGH THE INTESTINAL MUCOSA[1]

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It is the purpose of this paper to present quantitative data which provide further support for a physical hypothesis of intestinal absorption and secretion of fluid.

Previous work of the author (1) has indicated that absorption of fluid from the intestine may be effected by the energy of colloid osmotic forces exerted by proteins in the tissue fluids of the villi; for it was found, in the case of each of a series of animals, that the negative intra-intestinal pressure required to abolish absorption was equal to the colloid osmotic pressure of lymph from the lacteals of the same loop of gut. The author has suggested (2) that secretion of fluid by the bowel may result chiefly from the operation of simple physical mechanisms. Elevation of tissue pressure in the villi may result in ultrafiltration of tissue fluids through the epithelial membrane into the lumen of the gut. During secretion the villi are observed to be swollen, often to twice their resting diameter, and this appearance leads one to believe that tissue pressure becomes elevated as the result of filtration of fluid from the local capillaries. As a matter of fact, these vessels are congested, and the tonic contraction of the *muscularis mucosae* (which accompanies the secretion resulting from pilocaropine or from irritation of the mucosa) appears to offer obstruction to the drainage both of venous blood and of lymph. Although attempts have been made to measure tissue pressure in the mucosa by methods employed successfully in other tissues (3) local bleeding has always rendered these studies valueless. The present paper offers another, indirect, approach which seems to solve the problem; for the value of tissue pressure may be estimated in terms of capillary and osmotic pressures, under specified conditions.

The physical hypothesis of fluid transfer may be expressed in the form of the equation:

$$(1) \qquad \frac{dW}{dt} = k(TP - OP_t - IP)$$

In this formula, $\dfrac{dW}{dt}$, the rate of flow of fluid through the gut wall, is positive when fluid is secreted and negative during absorption. TP is the hydrostatic pressure and OP_t is the colloid osmotic pressure of the tissue fluids of the villi; IP is the intra-intestinal pressure; and k is the constant of proportionality. Since k varies with the area and permeability of the intestinal mucosa (factors which cannot be accurately measured) comparison of data from various animals is facilitated if the pressures can be so adjusted that neither absorption nor secretion occurs. Under these conditions $\dfrac{dW}{dt} = 0$, and the formula becomes:

$$(2) \qquad\qquad TP = OP_t + IP$$

When the gut is in the absorbing state—the villi being slender and the blood flow sluggish—it may be assumed that TP is close to atmospheric pressure. When $TP = 0$ the equation reduces to the form:

$$(3) \qquad\qquad OP_t = -IP$$

On the assumption that OP_t is the same as the colloid osmotic pressure of lymph from the lacteals, form (3) of the equation expresses the actual conditions required to balance the forces of secretion and absorption when the gut is in the absorbing state (1).

Except for the special case when secretion results from the application of excessive negative pressure to the contents of a loop of bowel (4), the mucosa of which is in the absorbing state, one finds that the villi and the whole thickness of the mucosa are swollen and turgid with fluid whenever secretion occurs. It may be assumed that the tissue pressure, TP, has a positive value when secretion is produced by mechanical or chemical stimulation of the mucosa, by cutting the mesenteric nerves, by intravenous injection of large amounts of saline, by injections of pilocarpine, or by procedures which elevate the portal venous pressure to a sufficient degree. Consequently the value of TP must be considered in any attempt at quantitative validation of the physical hypothesis of fluid transfer which includes a study of normal secretion. Since the value of TP cannot be obtained by direct measurement, it is fortunate that this variable can be eliminated from our equation by the substitution of equivalent quantities. It is evident that the physical hypothesis involves the factor of capillary filtration—the process by which fluid is supplied during secretion—and it must be assumed that, when intestinal absorption and secretion are balanced, capillary-absorbing and capillary-filtering forces are also in balance, provided fluid cannot escape from the villi by the lymphatic channels. Under these conditions:

$$(4) \qquad\qquad CP - TP = OP_b - OP_t$$

In this equation CP is the mean capillary pressure, TP is the tissue pressure, $OP_b - OP_t$ represents the effective colloid osmotic pressure: the difference between the total colloid osmotic pressure of the blood and the osmotic pressure of proteins in the tissue fluids. When the value of TP from equation (2) is substituted in equation (4) we obtain:

$$(5) \qquad\qquad CP - IP = OP_b$$

Equation (5) contains variables IP and OP_b which are easily measured, but since the capillary pressure in the villous circulation cannot be determined directly it is necessary to estimate CP indirectly. We have caused CP to vary by producing various degrees of congestion of the mesenteric veins. When properly applied to the mesenteric vessels, the crescent-
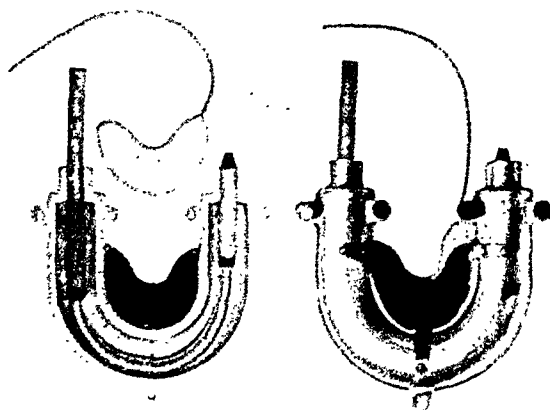
Fig. 1. Apparatus employed for congesting mesenteric vessels. Originally designed as a plethysmograph, the device was used, during this research, merely to provide support for parallel metal plates, between which were placed the mesentery of the isolated loop and the soft rubber bag used to produce congestion. The helical coil of aluminum wire, attached to its rubber-covered glass cannulae, is shown at the left. This device is placed inside the loop of gut, the ends of which are tied tightly over the cannulae.

shaped rubber bag, pictured in figure 1, may be blown up to compress the veins and lymphatics. Filtration of fluid from villous capillaries is doubtless accelerated by the resulting elevation of capillary pressure. The accumulating tissue fluid eventually swells the villi because its escape by way of the mesenteric lacteals is cut off at the point of application of the pressure bag.

It was found experimentally that, corresponding to any given intra-intestinal pressure—pressures slightly below atmospheric were employed throughout—there was a definite mesenteric venous pressure, measured by a water manometer connected to the rubber bag system, which exactly[2]

[2] The greatest error in determining each of the pressures which were measured (mesenteric venous, intra-intestinal, and colloid osmotic pressures) does not exceed

balanced the forces tending to cause absorption of fluid. Since venous pressure, during passive congestion, is probably not much lower than mean capillary pressure, it is not surprising to find, when the values of mesenteric venous pressure are substituted for CP in equation (5), that the equation actually expresses the conditions for the balancing of fluid-absorbing and fluid-secreting forces of the intestine. The graph of the data, shown in figure 2, indicates that fluid transfer through the gut wall will cease when mesenteric venous pressure and intra-intestinal pressure are so adjusted that their difference is equal to the total colloid osmotic pressure of the animal's blood serum. The line drawn at a slope of 45 degrees from the origin is the locus of points representing exact equality of $CP - IP$ and OP_b. It will be noted that the experimental points arrange themselves fairly accurately along the theoretical line.

The data were obtained from experiments on 10 dogs, anesthetized with sodium barbital (0.25 gram/kgm., injected intravenously). Single determinations were made on 7 animals. The eighth dog received an intravenous injection of 100 cc. of 30 per cent acacia solution, which elevated the osmotic pressure of the blood from its original value of 26 cm. of water to the high level of 42 cm. of water. The corresponding value of $CP - IP$ underwent a change of the order to be expected from the physical hypothesis of fluid transfer as expressed in equation (5). Another dog was bled until the osmotic pressure of its blood fell from 27 to 20 cm. of water. The tenth dog was bled four separate times, the osmotic pressure falling from the original value of 29 cm. to successive values of 24, 19, 14 and 10.5 cm. of water. In order to maintain the balance between absorbing and secreting forces it was necessary to diminish the congesting pressure at each stage, as indicated in the figure.

Intra-intestinal pressure was maintained, during these experiments, at from -3 to -5 cm. of water. The helical coil of aluminum wire, previously described (4) and pictured in figure 1, prevented collapse of the gut walls when internal pressure was reduced below atmospheric. The congesting pressures, required to balance absorbing and secreting forces, varied from 8 to 40 cm. of water. In one experiment intra-intestinal pressure was set at successive levels of -2, -6 and -11 cm. of water. The corresponding values of mesenteric venous pressure required to prevent fluid transfer in either direction were 24, 20 and 14 cm. of water, respectively. The values of $CP - IP$ were therefore 26, 26 and 25 cm. of water respectively: a fairly good check on the validity of the assumption that variations in venous pressure and intra-intestinal pressure are equally

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1.0 cm. of water. A change of less than 0.5 cm. of water in any of these pressures will produce a readily detectable change (0.01 cc. per minute or greater) in the rate of absorption or secretion.

effective in influencing the transfer of fluid, at the point of balance between secretion and absorption.

It has been observed that during congestion of the mesenteric veins and lacteals, the whole thickness of the gut wall becomes swollen, and fluid weeps constantly from the peritoneal surface of the loop. This fluid loss

Colloid Osmotic Pressure of Blood
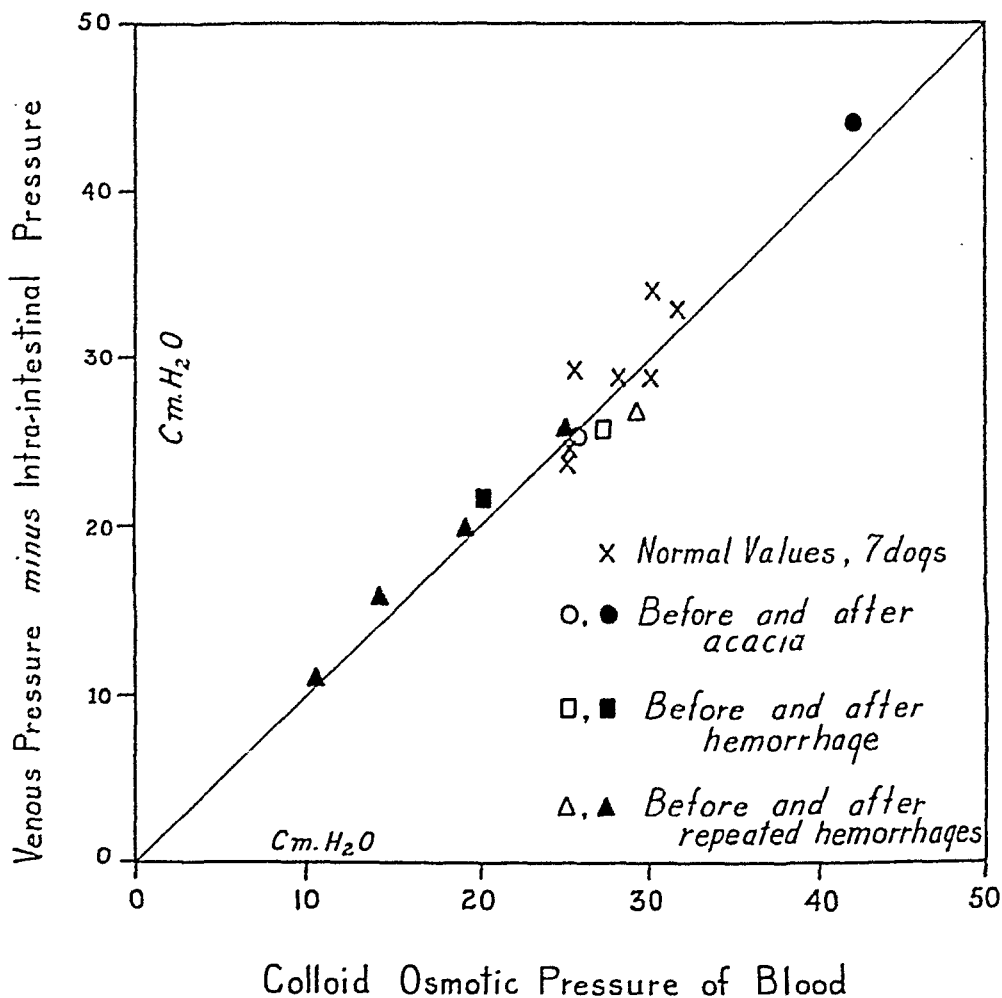
Fig. 2. Data indicating that when mesenteric venous and intra-intestinal pressures are adjusted to balance fluid-absorbing and secreting forces the difference of these values is approximately equal to the colloid osmotic pressure of the blood, over a wide range of values of the latter. The diagonal line is the locus of points representing exact equality of these variables, as demanded by theory.

presents possibilities for error in the analysis, for it has been tacitly assumed, in the discussion of the mathematical physics of the filtering-absorbing system, that the whole mechanism is enclosed in elastic, non-leaking walls. Consequently when we examine the graph of figure 2 it is not perhaps surprising that more points are found to lie above the theo-

retical line than below it. It seems evident that, in a slightly leaky system, capillary pressure must be maintained above the true point of equilibrium in order to provide for filtration at a rate equal to the losses from leakage. Most of this leakage, to be sure, may take place from the capillaries of the muscular coats and peritoneal surface, but it would be logical to believe that fluid can also escape by filtration from thin-walled lacteals as they penetrate the sub-mucosa and enter the muscularis. During the normal process of secretion such leakage presumably does not occur, since the veins and central lacteal of each villus are constricted at the base of the villus by the tonic contraction of the *muscularis mucosae.*

The error of estimating capillary pressure from venous pressure will tend to make the experimental points of figure 2 fall below the line. It is possible that this error in the negative direction may have become combined, in some instances, with the positive error mentioned in the preceding paragraph to give, by chance, the almost exact concordance of observed and theoretical values represented by the points which lie closest to the line.

Relation of intra-intestinal, congesting, and osmotic pressures to the rate of secretion of intestinal juice. If, at any constant value of intra-intestinal pressure, the congestion of the mesenteric veins and lymphatics is increased beyond the point of fluid balance, secretion will occur at a rate which attains a constant value, the magnitude of which is determined by the congesting pressure. A loop of bowel 20 cm. long may be caused, in this manner, to secrete fluid at a rate of 0.5 to 1.0 cc. per minute without becoming damaged; but at congesting pressures above 40 to 60 cm. of water, hemorrhage from the mucosa may complicate the experiment. Usually the effects of congestion are completely reversible; thus, upon release of the congesting pressure, the gut, within a few minutes, resumes absorption of fluid at precisely the rate observed during the period preceding the congestion.

If the congesting pressure is elevated in successive steps above the point of fluid balance, fluid is secreted at rates which increase geometrically rather than in direct ratio to the increments of venous pressure. Changes in intra-intestinal pressure at constant congesting pressure produce nearly proportional changes in the rate of secretion or absorption, but the constant of proportionality increases as the congesting pressure is increased. Stated in another way, the relation of fluid flow to intra-intestinal pressure is approximately linear, but the slope of the line increases geometrically as congesting pressure increases. It is possible that these deviations from proportionality are due to a progressive increase in the surface and a diminishing thickness of the epithelial membrane of the villi as they become progressively more swollen by increasing grades of congestion. There are other possibilities, but this explanation seems most

probable, for visual observation of the villi, which was made possible by the use of a special glass-covered chamber previously described (2), showed definitely that the villi did become swollen whenever the venous outflow from the loop was partially obstructed, and the degree of swelling paralleled the degree of venous congestion.

It has not seemed necessary to make special tests of enzymatic action to determine whether the juice put out by the mucosa as the result of venous congestion is normal succus entericus. In the first place, we are interested primarily in the exchange of fluid and not in its composition with respect to solutes. In the second place, Nasset and Parry (5) have found that the fluid which may be "sucked" into the gut by negative intra-intestinal pressure possesses the properties of normal intestinal juice; and there is no reason to believe that the "congestion juice" should differ from "suction juice" in any significant manner. The fluid which we have obtained was opalescent, and it contained no trace of blood.

As might be expected from the discussion so far, changes in colloid osmotic pressure of the blood are reflected in corresponding variations in the absorbing force of the gut, and in inverse changes in the rate of secretion. Thus, if an animal is bled and the volume of blood removed is replaced by saline, or if excessive amounts of saline are injected into the normal animal, without previous hemorrhage, secretion will start within a minute or two in a loop of bowel the intra-intestinal and congesting pressures of which have been previously adjusted to the point of fluid balance. In the case of the dog which suffered a single hemorrhage (200 cc.) the absorbing force, as measured by the intra-intestinal pressure necessary to balance the flow of fluid without venous congestion, fell from its original value of 19 cm. to 9 cm. of water.

DISCUSSION. Whether physical forces are or are not entirely responsible for the transfer of fluid through the intestinal mucosa, it is clear that variations in capillary, colloid osmotic, tissue and intra-intestinal pressures, each and severally, produce profound effects on the absorption and secretion of water. The fact that venous and lymphatic congestion of sufficient grade will invariably stop the absorption of water and that more severe degrees of congestion will cause significantly rapid losses of fluid into the lumen of the gut as well as into the peritoneal cavity are facts which are of importance for clinical medicine. The degree of congestion required to balance the forces of absorption—35 cm. of water is an average normal value of the colloid osmotic pressure of the blood of man—is probably not outside the range of values of venous pressure observed clinically in patients suffering from congestive heart failure, constrictive pericarditis, portal cirrhosis, etc. It would be well to point out, however, that values of venous or intra-intestinal pressure may not be used, in the equations which we have derived, unless these pressures are measured in relation to intra-abdominal pressure as the zero point.

The work of Herrin and Meek (6) on intestinal obstruction showed that distention of the bowel sets up a copious secretion resulting in important losses of fluid and salts from the body. Previous experiments of our own (unpublished) indicate that distention results in venous obstruction, and therefore it may be presumed that the secretion is due to the elevation of capillary and tissue pressures.

We have found that dogs which are very deeply anesthetized with barbital (0.35 gram per kilo) often show enlarged spleens, engorged mesenteric veins and portal pressures up to 45 cm. of water. When loops of bowel are isolated for study in such animals they invariably secrete continuously until the venous pressure is lowered to a normal level by the administration of a full dose of atropine, whereupon absorption only rarely fails to set in. The action of atropine to facilitate absorption of fluid has been noted by others (7). It is possible that the effect just described is due to some action of the drug on the hepatic sluice mechanism; but atropine also relaxes the outer gut musculature, an effect which should facilitate absorption in cases in which the tone of these muscles causes local venous congestion.

Objections to the physical hypothesis of intestinal fluid transfer have been raised by several writers. Some have insisted that absorption and secretion of fluid occur simultaneously; that changes of volume of solutions placed in the gut therefore represent the resultant effects of the two processes; and that, consequently, attempts to measure the rates of fluid absorption and secretion separately are doomed to failure. This pessimistic view appears to be based on the preconceived idea—no longer tenable—that living cells are unable to transport separate ions or molecules, but must move solutions en masse. The fact that water can be absorbed while salts and other blood constituents and enzymes are passing outward into the contents of the gut is no more difficult to explain by well known laws of diffusion than is absorption of tissue fluids through the walls of a blood capillary at the same time that a dye is diffusing out of the capillary into the tissue spaces.

The fact that the gut can absorb water from a hypertonic solution of NaCl has been considered as proof of the existence of special absorbing forces capable of operating against several atmospheres of osmotic pressure. But the laws of osmotic pressure tell us nothing whatsoever about the initial direction of movement of water from one solution to another except when the membrane separating them is absolutely impermeable to all solutes. Ions or molecules which are diffusing rapidly through a membrane may be expected to "carry water with them" in what appears to be the wrong direction. It was shown by Lazarus-Barlow in 1896 (8) that ox serum would absorb water from a 2 per cent NaCl solution across an artificial membrane which was permeable to the salt but impermeable to the serum proteins. Most physiologists realize that red blood cor-

puscles are quickly hemolyzed by hyperosmotic solutions of urea in water. Thermodynamic relations of this type of "anomalous" osmosis have been discussed by Shreinemakers (9).

Colloid osmotic forces have for years been considered inadequate to account for the absorption of water from blood serum or solutions of acacia. However, as Rabinovitch (8) and the author (unpublished) have shown, serum and acacia are absorbed at precisely the same rate as isotonic saline. This very exact equality of the absorption rates persists even at negative intra-intestinal pressures, and absorption of serum is retarded and eventually stopped by increasing degrees of negative pressure in the same manner and to the same extent as absorption of saline is affected by these relatively small changes in the applied force. These experiments, so far as they go, merely indicate that the proteins and acacia are, in some unknown manner, rendered completely incapable of exerting any colloid osmotic pressure, when they are brought in contact with the surface of the mucosa. The problem invites further study.

It has been suggested that the anesthesia or the negative intra-intestinal pressure employed in our experiments may abolish some of the special or selective absorbing or secretory forces of the gut, leaving unaffected those simple physical forces which the author has measured in previous studies; but the fact that "chloride impoverishment" (10) and absorption of serum—phenomena which involve special selective activity—have been shown to persist in our animals under the conditions prevailing in all of our experiments, would seem to rule out such objections to the physical hypothesis.

The author is well aware of the fact that the intestinal juice is not in equilibrium with the blood, so far as the various solute concentrations, pH, etc., are concerned (11, 12). It is clear that the mucosa performs osmotic work upon dissolved materials; but neither these phenomena nor the fact that the mucosa secretes mucus and enzymes need have any more bearing on absorption or secretion of water than the secretion of ammonia by the kidney has upon the process of glomerular filtration. There are no data which compel the belief that water is ever transferred by the gut against a true osmotic gradient.

It is urged, finally, that the scientific principle of economy of hypothesis favors the unitary and relatively simple theory proposed by the author to account for the transfer of fluid through the gut wall.

The remarkable experiments of Königes and Ottó (13), who find that central lacteal pressure in the villi of cats may attain values (24 to 25 mm. Hg.) considerably in excess of the colloid osmotic pressure of intestinal lymph (10 to 11 mm. Hg.), lend direct support to the physical hypothesis of intestinal secretion, and indicate that future study of the problems of fluid transfer may, after all, involve the use of direct micro-

pipette determinations of the capillary and tissue fluid pressures within the villi.

SUMMARY

1. Moderate congestion of the mesenteric veins and lymphatics produces slowing of the rate of absorption of isotonic saline from isolated loops of the small intestine. At a definite congesting pressure absorbing and secreting forces may be exactly balanced. Higher grades of congestion produce secretion of intestinal juice, the rates of secretion increasing geometrically as mesenteric venous pressure is increased above the value required to abolish absorption.

2. Analysis of the physical hypothesis which has been proposed to account for intestinal absorption and secretion of fluid, indicates that absorbing and secreting forces should be neutralized when the difference between mean capillary pressure in the villi and intra-intestinal pressure is equal to the colloid osmotic pressure of the animal's blood plasma. When venous pressure is used as the measure of capillary pressure the equation fits the data obtained experimentally.

3. Some objections, commonly raised against physical hypotheses of intestinal transfer of fluids, are discussed.

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THE SIZE OF THE EXTRACELLULAR FLUID COMPARTMENT BEFORE AND AFTER MASSIVE INFUSIONS

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The idea seems to have been widely accepted that certain substances amongst which are chlorides and sulfocyanides can be used to estimate the size of the extracellular fluid compartment of the body as a whole or the size of the extracellular compartment of its various organs (1, 2, 3). The principle of the estimation involves two assumptions: 1, that such substances do not penetrate the cells, and 2, that in the fluids outside the cells they undergo and are maintained in uniform distribution. It can be easily shown that chlorides and sulfocyanides do enter the erythrocytes of the blood stream (1), but the adult red corpuscles are not true cells morphologically in that they contain no nuclei and there is reason for believing that their behavior is not necessarily that of true cells. On the other hand, experimental findings have shown with remarkable uniformity a constant fraction of the total body substance not invaded by chlorides and sulfocyanides (approximately 70 per cent by volume) and it has been argued that this can only represent that portion composed of cells.

The experiments now being reported have made use of the above assumptions in attacking the general problem of the physiologic response to massive infusions of approximately isotonic solutions of sodium chloride and glucose concerning which several reports from this laboratory have already been published (4, 5, 6). The present investigation is concerned with the estimation, by the chloride and sulfocyanide methods, of the normal size of the total extracellular fluid compartment of the body, the extracellular compartment of its component organs, and the manner and extent of modification of the size of these compartments by massive infusions.

METHODS AND CALCULATIONS. As in previous investigations, cats were used as experimental animals. They were anesthetized by injecting

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intraperitoneally either dial[1] (60 mgm./kgm. body weight) or phenobarbital (40 mgm./kgm. body weight). A suitable accurately weighed quantity of sulfocyanide (usually about 140 mgm./kgm. body weight) was then given by vein and 30 minutes were allowed for uniform distribution throughout the sulfocyanide available volume of the animal. At the end of this time 10 ml. of blood were withdrawn from the carotid artery and set aside for subsequent sulfocyanide (7) and chloride (8) determinations. Control animals (not infused) were immediately sacrificed by asphyxia and their tissues taken for analysis. The control group comprises 14 animals.

In the case of the NaCl solution the amount given was such as to give a total infused volume of 400–550 ml./kgm. initial body weight and a retained volume of 375 ml./kgm. initial body weight. Glucose solutions were given in such amounts as to give a total infused volume of 300–475 ml./kgm. initial body weight and a retained volume of 275 ml./kgm.

TABLE 1

Volume of extracellular water in the cat on the basis of sulfocyanide distribution and total chloride content

EXPERIMENT	WEIGHT	AVAILABLE WATER IN ML./KGM. CAT	
		Sulfocyanide	Chloride
	kgm.		
A	1.98	288	316
B	1.65	288	320

initial body weight. These amounts represent volumes certainly not lethal but definitely approaching lethal values.

Thirty minutes were allowed after the termination of the infusion for uniform distribution of the infusate. Ten milliliters of blood were then withdrawn from the carotid artery and were set aside for subsequent chloride and sulfocyanide analysis. The animals were killed by asphyxia. Various organs were removed promptly, blotted to remove excess blood and accurately weighed. These were then dried to constant weight in an oven maintained at 105°C. and their dry weight determined. The fat was removed by extraction with ether and the fat free weight determined. The dried, fat free tissue was subsequently dissolved in normal KOH solution from which aliquot portions were taken for chloride (9) and sulfocyanide (10) analysis.

In all cases excreta (feces, urine and vomitus) were collected and analyzed for chlorides and sulfocyanide.

[1] Supplied by courtesy of Ciba Pharmaceutical Products, Inc. Lafayette Park, Summit, N. J.

In two additional experiments after determining the sulfocyanide available volume of the animals, the whole cat was dissolved in normal KOH solution. The chloride content of the dissolved animal was determined and the chloride available volume calculated. These volumes were then compared (table 1).

The following calculations have been made and tabulated:

Sulfocyanide available water of the uninfused cat in ml./kgm. body weight (AW_S):

$$\text{(1)} \quad \frac{\dfrac{\text{mM sulfocyanide injected/}}{\text{kgm. body weight}} - \dfrac{\text{mM sulfocyanide excreted/}}{\text{kgm. body weight}}}{\text{mM sulfocyanide/ml. serum}}$$

Sulfocyanide available water of the infused cat in ml./kgm. final [2] body weight (AW_S');

$$\text{(2)} \quad \frac{\dfrac{\text{mM sulfocyanide injected/}}{\text{kgm. final body weight}} - \dfrac{\text{mM sulfocyanide excreted (and in peritoneal}}{\text{exudate)/kgm. final body weight}}}{\text{mM sulfocyanide/ml. serum after infusion}}$$

Chloride available water of the infused cat in ml./kgm. final body weight (AW_{Cl}');

$$\text{(3)} \quad \frac{\left\{\begin{array}{l}\text{total sulfocyanide} \\ \text{available water in} \\ \text{ml./kgm. original} \\ \text{body weight}\end{array}\right\} \times \begin{array}{l}\text{serum chloride of} \\ \text{the uninfused cat} \\ \text{in mM/ml.}\end{array} + \begin{array}{l}\text{[3] infused chloride} \\ \text{retained in mM/kgm.} \\ \text{original body weight}\end{array}}{\text{serum chloride of the infused cat in mM/ml.}}$$

Sulfocyanide available water of individual organs in ml./kgm. final body weight;

$$\text{(4)} \quad \frac{\text{sulfocyanide in mM/kgm. organ}}{\text{sulfocyanide in mM/ml. serum}}$$

Chloride available water of individual organs in ml./kgm. final weight;

$$\text{(5)} \quad \frac{\text{chloride in mM/kgm. organ}}{\text{chloride in mM/ml. serum}}$$

Calculated sulfocyanide available water of individual organs of infused cats in per cent total water following infusion:

$$\text{(6)} \quad \frac{\left\{\begin{array}{l}\text{sulfocyanide available water} \\ \text{in ml./kgm. original weight}\end{array} + \begin{array}{l}\text{total increase in water con-} \\ \text{tent in ml./kgm. original} \\ \text{weight.}\end{array}\right\} \times 100}{\left\{\begin{array}{l}\text{1000 + increase in water content} \\ \text{in ml./kgm. original weight}\end{array}\right\} \times \begin{array}{l}\text{water content in ml./gm. in-} \\ \text{fused organ}\end{array}}$$

Calculated chloride available water of individual organs of infused cats in per cent total water following infusion:

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[2] Final body weight, as used in this study, represents the original weight to which is added the volume of fluid infused less the volumes of fluid excreted by all excretory organs and that volume of fluid found free in the peritoneal cavity at the end of infusion.

[3] Chloride lost by excretion or passing into water in the peritoneal cavity during glucose infusion must be subtracted.

(7)

$$\dfrac{\left\{\begin{array}{c}\text{chloride available water in}\\ \text{ml./kgm. original weight}\end{array} + \begin{array}{c}\text{total increase in water content in ml./kgm. original weight}\end{array}\right\} \times 100}{\left\{\begin{array}{c}1000 + \text{increase in water content}\\ \text{in ml./kgm. original weight}\end{array}\right\} \times \begin{array}{c}\text{water content in ml./gm.}\\ \text{infused organ}\end{array}}$$

I. *The size of the chloride and sulfocyanide available volumes in the normal cat.* The total chloride available water of the cat is slightly greater than the sulfocyanide available water (table 1). This becomes especially

TABLE 2

Sulfocyanide and chloride available volumes of the organs in normal cats

ORGAN	NORMAL CHLORIDE VALUES					NORMAL SULFOCYANIDE VALUES				
	Experiment 3 Chloride available water			Average chloride available water (12 experiments)		Experiment 3 Sulfocyanide available water			Average sulfocyanide available water (12 experiments)	
	Chloride (mM/kgm. organ)	Ml./kgm. organ	Per cent total	Ml./kgm. organ	Per cent total	Sulfocyanide (mM/kgm. organ)	Ml./kgm. organ	Per cent total	Ml./kgm. organ	Per cent total
Blood serum†	127.0					5.70				
Gastrocnemius	19.7	155	20.8	124	16.2	0.96	169	22.6	162	21.2
Biceps femoris	20.4	161	21.2	132*	17.2	1.03	180	24.2	137*	17.9
Triceps brachii	19.0	150	19.5	133*	17.5	1.00	175	23.1	134*	17.7
Temporalis	20.6	162	22.0	153*	20.6	1.01	177	24.4	168*	22.6
Spinotrapezius	27.2	215	28.2	200*	26.0	1.33	233	30.6	202*	26.3
Sternomastoideus	41.3	325	44.5	248*	32.4	1.80	317	43.5	240*	31.7
Diaphragm	53.4	423	56.5	293*	38.0	2.70	475	63.5	329*	42.9
Lateral abdominal wall muscle	44.7	353	45.6	351*	45.7	1.83	322	41.7	359*	46.9
Rectus abdominis	60.0	473	60.0	375*	49.0	2.58	455	57.9	344*	45.0
Cardiac muscle	41.0	322	42.0	302	38.2	1.94	342	44.3	329	41.5
Liver	28.7	227	31.4	285	40.0					
Brain (cerebrum)	34.7	274	31.4	325	47.8	0.46	81	9.3	87	10.1
Duodenum	50.7	400	51.5	385	48.6	1.48	260	33.7	326	41.1
Colon	57.0	451	58.5	416	53.2	2.96	519	67.5	447	57.1
Spleen	52.6	414	54.0	426	54.4	2.38	420	54.5	374	47.8
Pancreas	81.5	642	84.5	407	52.0	1.92	336	44.2	327	41.8
Stomach	79.2	623	76.6	548	68.2	2.88	507	62.0	467	58.2
Lung	77.0	607	77.0	576	72.5	2.17	382	48.5	401	50.3
Kidney	97.5	767	98.5	586	75.0	2.96	519	66.0	447	57.2
Skin	99.5	785	115.5	684	102.0	4.52	793	117.0	602	90.0

* Average determinations from four experiments.

† Average chloride 127.0 mM/kgm. serum. Average sulfocyanide 5.96 mM/kgm. serum.

apparent when the various organs are separately analyzed and their chloride and sulfocyanide available water volumes are compared (table 2). The volume of water available for the distribution of chloride and sulfocyanide in a given organ varies somewhat from animal to animal. The distribution in the skin indicates a volume available to both of these substances equal to the total water content (total water content 67.03 ml./100 grams; calculated NaCNS available volume 60.2 ml./100 grams; calculated chloride available volume 68.4 ml./100 grams). The brain

has a chloride available water volume much in excess of that available to sulfocyanide. This apparently results from the slow penetration of sulfocyanide into the cerebrospinal tissue spaces, the water of which contains a slightly higher chloride concentration than that of the blood plasma. The general inertia of the cerebrospinal fluid to changes in the composition of the plasma following infusion is quite clearly demonstrable

TABLE 3a

Volume of chloride and sulfocyanide available water following infusion with 5 per cent glucose solution

ORGAN	EXPERIMENT 15 CHLORIDE AVAILABLE WATER			AVERAGE CHLORIDE AVAILABLE WATER (5 EXPERIMENTS)			EXPERIMENT 15 SULFOCYANIDE AVAILABLE WATER			AVERAGE SULFOCYANIDE AVAILABLE WATER (5 EXPERIMENTS)		
	Chloride (mM/kgm. organ)	Ml./kgm. organ	Per cent total	Ml./kgm. organ	Per cent total	Calculated water*	Sulfocyanide (mM/kgm. organ)	Ml./kgm. organ	Per cent total	Ml./kgm. organ	Per cent total	Calculated water
Blood serum‡	60.2						3.50					
Gastrocnemius	10.0	167	21.8	164	21.2	19.3	1.05	300	39.0	339	44.0	24.1
Biceps femoris	11.2	186	24.0	169	21.9	20.7	1.01	289	37.6	257	33.2	21.4
Triceps brachii	13.2	220	28.8	174	22.5	23.4	0.89	255	33.4	336	43.6	23.6
Temporalis	20.0	332	42.5	240	31.0	32.7	1.53	438	56.0	412	52.0	34.4
Spinotrapezius	18.6	309	40.0	288	37.0	29.8	1.23	351	44.5	443	57.1	30.0
Sternomastoideus	31.7	526	66.5	458	57.0	48.3	2.72	777	97.2	543	67.7	47.5
Diaphragm	21.1	350	43.0	358	44.5	49.5	1.37	392	48.0	485	60.5	52.9
Lateral abdominal wall muscle	16.6	276	35.0	285	36.0	52.1	1.32	377	48.0	497	62.8	53.3
Rectus abdominis	22.4	372	47.2	373	47.8	54.0	1.73	495	62.8	458	58.7	50.0
Cardiac muscle	22.6	374	46.7	365	44.8	45.8	1.57	449	55.6	462	56.9	48.8
Liver	20.4	336	41.5	364	46.5	58.4						
Brain	35.7	592	69.0	573	67.0	35.6	0.64	183	21.3	220	25.7	6.8
Duodenum	39.4	654	81.1	565	70.1	52.7	2.70	770	94.5	655	81.5	46.0
Colon	35.4	588	68.4	650	78.0	66.4	2.86	819	95.0	810	93.5	69.2
Spleen	37.2	618	78.7	590	76.9	43.3	1.87	535	68.4	563	73.3	37.0
Pancreas	35.2	585	64.2	825	91.0	83.0	1.79	511	56.4	634	69.7	79.5
Stomach	39.2	651	74.3	755	88.0	78.0	2.50	715	81.8	563	65.8	71.9
Lung	34.9	580	65.4	550	62.7	85.8	2.58	738	83.0	768	87.5	73.6
Kidney	26.2	435	51.4	497	58.3	84.5	1.44	412	48.7	483	56.6	73.6
Skin	46.2	767	110.0	763	101.0	102.2	4.22	1200	173.0	980	130.0	94.0

* Obtained from equation 7.
† Obtained from equation 6.
‡ Average chloride 60.8 mM/kgm. serum. Average sulfocyanide 4.93 mM/kgm. serum.

and will be discussed presently. After the brain, the organs showing the greatest difference between chloride and sulfocyanide available water are lung, kidney and stomach. The kidney, under normal conditions, excretes very small quantities of sulfocyanide during the first few hours following injection. The concentration of this substance in the tubules must be relatively less than the concentration of chloride and would thus give a much lower volume of distribution than would be indicated by the

chloride content of the organ.   The difference between the sulfocyanide and chloride available water in the stomach would indicate that about 14 per cent of the total chloride of this organ is in some of the gastric cells. Manery and Hastings (12) have recently reported the results of separate analyses of fundic and pyloric mucosa.   They report similarly high chloride values for these tissues.   Muscles differ a great deal from one

TABLE 3b

Volume of chloride and sulfocyanide available water following infusion with 1 per cent sodium chloride solution

ORGAN	EXPERIMENT 16 CHLORIDE AVAILABLE WATER			AVERAGE CHLORIDE AVAILABLE WATER (5 EXPERIMENTS)			EXPERIMENT 16 SULFOCYANIDE AVAILABLE WATER			AVERAGE SULFOCYANIDE AVAILABLE WATER (5 EXPERIMENTS)		
	Chloride (mM/kgm. organ)	Ml./kgm. organ	Per cent total	Ml./kgm. organ	Per cent total	Calculated water*	Sulfocyanide (mM/kgm. organ)	Ml./kgm. organ	Per cent total	Ml./kgm. organ	Per cent total	Calculated water†
Blood serum‡	136.5						2.99					
Gastrocnemius	25.0	183	23.8	241	30.7	27.3	0.57	191	25.0	229	29.2	31.7
Biceps femoris	25.4	186	23.8	217	27.4	28.4	0.62	207	25.7	215	27.2	28.9
Triceps brachii	30.7	225	29.0	254	32.3	29.6	0.67	224	28.9	250	31.8	29.7
Temporalis	43.6	320	41.6	345	43.6	39.4	0.92	307	40.2	329	41.5	40.9
Spinotrapezius	41.2	302	38.5	363	45.8	36.0	0.77	257	32.6	340	43.0	36.2
Sternomastoideus	64.5	472	58.5	462	56.8	52.0	0.82	274	33.5	412	50.7	51.3
Diaphragm	52.5	384	48.0	437	53.4	53.7	1.23	411	51.5	382	46.8	57.2
Lat. abd. wall muscle	53.0	388	48.8	392	49.0	54.2	1.17	392	49.0	410	51.5	54.8
Rectus abdominis	61.4	450	56.0	470	59.5	56.6	1.22	409	51.0	462	58.5	53.0
Cardiac muscle	54.9	402	50.5	435	53.5	45.7	0.99	331	41.3	368	45.3	48.9
Liver	38.6	283	37.4	332	43.7	52.6						
Brain	40.9	300	34.6	341	39.5	39.2	0.66	220	25.6	172	20.0	12.3
Duodenum	77.1	564	69.2	520	63.9	55.0	1.62	541	67.0	529	65.0	48.6
Colon	87.0	637	75.0	662	78.0	70.5	1.73	580	68.5	604	71.0	73.0
Spleen	52.4	384	49.0	459	59.3	50.3	1.32	440	56.9	464	60.0	43.2
Pancreas	106.0	776	82.5	884	94.0	86.7	1.47	492	61.2	469	50.0	84.5
Stomach	107.0	785	85.6	859	96.0	85.0	1.78	593	73.5	694	77.5	79.8
Lung	89.5	657	77.6	725	84.0	82.8	1.27	425	50.5	468	54.2	69.0
Kidney	93.1	681	79.0	650	76.0	85.8	1.41	472	54.5	395	46.0	75.0
Skin	93.6	688	96.2	845	103.8	101.0	2.54	850	120.0	904	111.0	95.1

* Obtained from equation 7.
† Obtained from equation 6.
‡ Average chloride 137.0 mM/kgm. serum.   Average sulfocyanide 4.0 mM/kgm. serum.

another.   They may be divided into two general groups, *a*, the thick muscles such as the biceps femoris or gastrocnemius having low volumes of distribution which are of similar magnitude from animal to animal, and *b*, the thin muscles having generally quite large volumes of distribution which are somewhat variable from animal to animal.   In both groups, however, the sulfocyanide and chloride available water volumes of normal cats are quite similar if not identical.

II. *The size of the chloride and sulfocyanide available volumes following massive infusion of 1 per cent sodium chloride or 5 per cent glucose solution.* Water and chloride, infused as a 1 per cent sodium chloride solution, is retained for the most part in the chloride and sulfocyanide available (extracellular) water. A comparison of the volume of water retained with the increase in size of the chloride and sulfocyanide available water (table 4) shows almost complete agreement on the basis of chloride distribution and a small but consistent increase in the size of the volume of water available to sulfocyanide. Such sulfocyanide values would seem to be most reasonably explained on the hypothesis that some of the cells become permeable to this ion as a result of infusion. Skin is of interest in this connection. In the normal animal, chloride and sulfocyanide were found distributed through a volume of water equal to the total water content of the skin. Following infusion with sodium chloride solution, sulfocyanide was present in amounts indicating an average water content of 90.4 ml./100 grams whereas the total water content found was only 81.0 ml. which suggests a binding of sulfocyanide in some manner by the skin. Similar behavior seems likely to a limited extent in the case of some of the other organs.

Following the infusion of large amounts of 5 per cent glucose solution the volume of water through which sulfocyanide is distributed is more variable. A comparison between the volume of water infused and the volume of water available for the solution of sulfocyanide gives values which indicate a range of variation from no difference at all to as great a difference as 411 ml./kgm. of cat in one instance (table 4). This difference was associated with corresponding differences in behavior of the various organs. When the sulfocyanide available water is in excess of the volume of infused water retained, the individual organs show a corresponding increase in the amount of the sulfocyanide available water. It thus appears that the permeability of the cell may be increased with respect to the sulfocyanide ion as a result of massive infusions of 5 per cent glucose. On the contrary, there is no appreciable increase in the permeability of the cell to chloride as a result of glucose infusions even though the response is somewhat more variable than in those animals infused with 1 per cent sodium chloride solution. It can be concluded from chloride analyses that the glucose solution infused was confined, for the most part, to the extracellular compartment of the body.

A summary of the data obtained by analysis of the various organs following infusion is presented in table 3 a and b. There is close agreement between the volume of sulfocyanide and chloride available water for any given muscle following infusion with 1 per cent sodium chloride solution. The increase in the water content is for the most part confined to the extracellular compartment. After infusion with 5 per cent glucose solu-

tion, analysis for chloride indicates that most of the increase in water takes place in the extracellular compartment. The volumes of sulfocyanide available water are somewhat variable and for that reason the averages presented may be misleading. In one instance the injected sulfocyanide left the circulation in large amounts and in this instance the available volumes were much greater in the various muscles than the observed increases in water would indicate.

Chloride analyses of the livers of perfused animals suggest that the cells of this organ are normally impermeable to the chloride ion. Truax (13) has recently reported a comparison between the histological extra-

TABLE 4

The effect of 5 per cent glucose and 1 per cent sodium chloride infusions on the sulfocyanide and chloride available water in cats*

CAT NUMBER	1 (AW_s)	2 Water Retained	3 Calculated $\frac{(AW'\dagger)}{(1+2)1000}$ $\frac{}{(2+1000)}$	4 Observed (AW'_s)	5 Difference $(4-3)$	6 (AW'_{cl})	7 Difference $(6-3)$
			5 per cent glucose infusion				
15	300	225	429	500	71	415	−14
18	348	182	448	490	42	530	82
20	352	131	425	836	411	459	34
21	316	164	411	387	−24	408	−3
23	389	233	504	570	66	578	74
			1 per cent sodium chloride infusion				
16	232	253	386	442	56	404	18
17	327	370	512	530	18	496	−16
19	389	253	512	584	72	518	6
22	450	230	553	624	71	552	−1
24	462	264	575	657	82	557	−18

* All values in milliliters per kilogram of cat.

cellular space and the chloride available water of the liver of the white rat. He finds these to be quantitatively equal, thus giving the chloride an exclusively extracellular distribution. The calculated increase in the water in the livers of perfused cats, assuming all of the increase to be in the extracellular water, is in all instances greater than the observed increase. Approximately 40 per cent of the water taken up by this organ as a result of infusion seems to be taken into the intracellular compartment. Sulfocyanide analyses of the liver could not be performed due to interfering colors which could not be removed.

The stomach appears to contain intracellular chloride. Following the

infusion of 5 per cent glucose solution there is an average increase of 13.2 ml./100 grams in the water content of this organ whereas the chloride distribution would indicate a gain of 23.4 ml./100 grams. There must have been cellular chloride not readily available to the extracellular fluids. That the stomach readily takes up sulfocyanide in glucose infused animals is indicated by an average available volume of 108 ml. Fluid collected from the stomachs of these animals contained sulfocyanide in approximately the same concentration as the blood plasma. Following infusion of 1 per cent sodium chloride solution, chloride analysis gave an average increase in water of 31.1 ml./100 grams whereas the actual increase was only 22.4 ml./100 grams. Inasmuch as the cells of the stomach take up some chloride and sulfocyanide the estimation of the ratio of extracellular to intracellular water on the basis of the distribution of these substances is not possible. The small and large intestine behave in a manner essentially similar to the stomach.

Chloride distribution in the pancreas suggests, as above, that there is a small amount of chloride not readily available to the extracellular water. It would seem however, on the basis of the distribution of this ion, that most of the gain in water is in the extracellular compartments. Sulfocyanide analysis of the pancreas of saline infused animals gives a volume approximately one-half that for chloride distribution. This would suggest either an increase in water and chloride content of the cells or the existence of some extracellular water not readily available to the sulfocyanide ion.

An accurate analysis of available volumes of the kidney is difficult because of the uncertain amount of water, chloride and sulfocyanide in the tubules. In sodium chloride infused animals however, the increase in water content, if confined to the extracellular water, would amount to 15 ml. whereas the chloride content indicates an average increase in volume of only 6.1 ml. Inasmuch as the urine of the animals contained chloride in higher concentration than the plasma, the results cannot be explained on the basis of a salt poor fluid. There must have been an increase in the size of the cellular compartment.

The spleen did not show an increase in water as a result of infusion. This may not correctly represent the conditions in this organ inasmuch as asphyxia which causes splenic contractions was used to terminate the experiments. However, it is of interest to note that the chloride and sulfocyanide content indicates a larger amount of water than was actually found present. In the case of 5 per cent glucose infusion this was quite marked. The chloride and sulfocyanide concentration of the blood being low at the end of infusion with glucose solution, the difference between theoretical and observed values would tend to be exaggerated if these substances were not readily available to the extracellular fluids. Here, as previously suggested, there is some evidence of "binding" of these ions.

The cerebrum is of particular interest in that the sulfocyanide available water is normally very small (av. 8.7 ml./100 grams whereas the chloride available water is quite large (av. 32.5 ml./100 grams). The sulfocyanide available water is small compared to the total water available, i.e., the vascular water and cerebrospinal fluid water. The changes in the sulfocyanide content of the cerebrospinal fluid do not run parallel to changes in the composition of the plasma. The sulfocyanide concentration of this fluid is much lower than the concentration in the plasma, within the time limits of our experiments. This difference appears to result from the unique nutritive system of this organ. The low plasma chloride resulting from infusion of 5 per cent glucose solution does not materially reduce the chloride content of the cerebrospinal fluid. If a large part of the fluids of the extracellular spaces of the brain behave like the cerebrospinal fluid, values such as reported here are to be expected. The infusion of 1 per cent sodium chloride elevates the plasma chloride which in turn causes an increase in the chloride content of the cerebrospinal fluid and the cerebrum. The chloride excess of the plasma appears to diffuse readily into the chloride available water of the brain but very slowly leaves this water to pass back into the blood stream when the chloride concentration of the latter is lower. The observed decrease in the sulfocyanide available water was due, for the most part, to the decrease in the sulfocyanide concentration of the blood plasma resulting from infusion. The sulfocyanide which had passed into the cerebrospinal fluid before infusion with 1 per cent sodium chloride is little influenced by the changes in the sulfocyanide concentration of the blood plasma resulting from infusion.

Reference has previously been made to the skin. In a few of the perfusion experiments, edematous subcutaneous tissue was dissected away and was separately analyzed. This invariably gave a value higher than the epithelial layers overlying it. In one glucose infused animal the skin was found to have 69.7 ml./100 grams of chloride available water whereas analysis of the subcutaneous tissues gave a value of 102 ml./100 grams. Sodium chloride infusion resulted in skin values of 91.0 ml. and 95 ml. respectively for sulfocyanide and chloride available water whereas analysis of edematous subcutaneous tissues gave values of 125 ml. and 120 ml./100 grams on the basis of sulfocyanide and chloride content. The high skin values obtained would appear to result from the "binding" of chloride and sulfocyanide by their subcutaneous tissues rather than by the epithelial elements.

DISCUSSION. Recent studies of the total chloride (2) and sulfocyanide (1) available volumes of various species of animals give a normal range of 25 to 45 per cent of the body weight. Our observations on the cat gives similar volumes of distribution for these two substances. Comparison

of the sulfocyanide and chloride available volumes in the same animal indicates a slightly greater available volume for the latter. This difference we believe to be due to "bound" chloride in some tissues. This possibility is suggested by the work of Amberson, Nash, Mulder and Binns (3) and more recently by the investigations of Manery and Hastings (12).

Several investigators have attempted to determine the extracellular-intracellular water ratio for some organs of the animal body (Harrison, Darrow and Yannet (2) chloride available volumes of the rabbit, dog and monkey; Wallace and Brodie (11) the chloride and bromide available volumes of the dog and the chloride, bromide and sulfocyanide available volumes of the various parts of the central nervous system of this animal; Manery and Hastings (12) the sodium and chloride available volumes of the rat and rabbit). The available volumes reported for any organ agree in a general way with the values here reported for the normal cat and suggest a similar distribution of these ions in the mammals studied. In some instances values have been given for skeletal muscle or intestine without specifying the particular muscle or part of the intestine which was taken for analysis. We have found the amount of chloride and accordingly the chloride available water of muscles to vary widely. The chloride available water of the duodenum is, on the average, less than that of the colon. In the uninfused cat there is reasonably good agreement between the volumes of water available to chloride and sulfocyanide in skeletal muscle. A comparison of the values for these two substances in other organs shows similar magnitudes of distribution in all organs except the cerebrum. The investigations of Wallace and Brodie (11) suggest that a similar difference in available water would be found for the whole central nervous system.

A comparison between the volume of water retained and the increase in the amount of the chloride available water following infusion of 5 per cent glucose and 1 per cent sodium chloride suggests that most of the fluid infused is retained in the extracellular compartment but analysis of individual organs shows that this is not strictly the case. The stomach takes up chloride to a greater extent than water in the case of infusion with 1 per cent sodium chloride solution and does not readily lose chloride after infusion with 5 per cent glucose solution. The cerebrum readily gains chloride as a result of 1 per cent sodium chloride infusion and very slowly loses chloride when the concentration in the blood is lowered by infusion with 5 per cent glucose solution. In neither case does the brain gain appreciable amounts of water. The skin appears to contain salt and water in comparable amounts but separate analysis of the epithelial portion and the subcutaneous tissue suggests that the latter is most important in binding

both chlorides and sulfocyanide, containing these substances in exceedingly large amounts. Manery and Hastings (12) have likewise found that connective tissues may "bind" large amounts of sodium and chloride.

Our data suggest that the thick muscles and possibly the liver most nearly have a strictly extracellular distribution of chloride and sulfocyanide and that infusion of 5 per cent glucose may alter the distribution of the latter ion.

SUMMARY

1. Chloride and sulfocyanide are distributed through a volume of fluid greater than that contained in the extracellular compartment.

2. These substances are normally limited to the extracellular spaces of skeletal muscle. Chlorides appear to be confined to the extracellular compartment of the liver.

3. There is some intracellular chloride in the stomach, small and large intestine, spleen and pancreas.

4. Chloride and sulfocyanide are distributed through a volume of fluid approximately equal to the total water content of the skin.

5. Infused 5 per cent glucose or 1 per cent sodium chloride solution is retained for the most part in the extracellular compartment.

6. Infusion of 5 per cent glucose may greatly increase the permeability of the cells of certain tissues and organs to sulfocyanide.

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POST-TETANIC POTENTIATION AND SUPPRESSION IN MUSCLE

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The literature concerning the nature of the augmenting or potentiating effect of an indirect tetanus on the response of a muscle to subsequent single nerve volleys is conflicting. Guttman *et al.* (1) found no potentiated response after a direct tetanus in the curarized frog muscle while Brown and v. Euler (2) found it in cat muscle. The magnitude of the muscle action-potential accompanying the potentiated twitch is less than that of the last twitch before the tetanus in the normal cat (2) and in the normal fowl (4), whereas in the partially curarized cat muscle it is greater (2). Brown and v. Euler think that in the normal cat muscle the potentiated twitch is the result of increased tension in each muscle fiber and that this increase is due to an accumulation of potassium ions outside of the muscle fiber as a result of tetanization. On the other hand, they believe (2) that in the partially curarized cat muscle the potentiated twitch is due to the response of more muscle fibers to the nerve volley and that this is due to a mobilization of potassium at the myoneural junction. In normal fowl muscle the potentiated twitch is likewise supposed (4) to be due to an increased number of muscle fibers responding, but this increase is not ascribed to a mobilization of potassium. Rosenblueth and Morison (3) and Feng *et al.* (5) explain potentiation in the normal cat as due to a mobilization of potassium at the myoneural junction, but Feng (6) believes that in the toad mobilization of acetylcholine brings it about, while Guttman *et al.* (1) think that it is due either to acetylcholine or to adrenine.

It is the object of this paper to attempt to resolve this conflict of interpretations and more particularly to answer the questions of Bronk and Brink concerning post-tetanic potentiation (7, p. 402): "Why a sympathetic ganglion should resemble a partially curarized muscle and whether it is indeed the same type of mechanism have not yet been determined." This attempt involves a study of the muscle and nerve action-potentials simultaneously and a reëxamination of potentiation during curarization and in the presence of potassium.

METHODS. The sciatic nerve and gastrocnemius muscle of *Rana pipiens* were dissected out in the usual way and placed in a beaker of amphibian Ringer's solution

for from $\frac{1}{2}$ to 2 hours before using. Sometimes the preparations were kept 24 hours in the refrigerator at 3 to 4°C. before using; these preparations were usually less excitable than fresh preparations. The action-potentials of the nerve and the response to direct stimulation in the latter preparations seemed normal, so that apparently the myoneural junction deteriorates more quickly than either the muscle or nerve fibers.

The preparations were then placed in a modified Keith Lucas bath. The modification consisted of a hard rubber block which replaced the end of the bath opposite the lever attachment. Five holes, 2 to 3 mm. in diameter and 6 to 7 mm. deep, were drilled along the block. The holes were connected by a trough 2 mm. wide and 3 mm. deep. The nerve was placed in the trough which was packed with white vaseline and the holes were filled with Ringer's solution. The knee joint was fixed and the tendon attached to the lever which was free weighted and which magnified 8 times. Mechanical records were made on smoked paper fastened to a spring-driven kymograph.

Chlorided silver electrodes attached to plugs in a bakelite panel were lowered into the holes in the block. The first two delivered make induction shocks from a coreless coil specially wound. This coil was driven by a special tube circuit which was synchronized with the sweep circuit of the oscillograph. For direct stimulation of the muscle a thyratron circuit was used without any induction coil. Both circuits could deliver shocks anywhere from 1 per second to 450 per second. The third electrode was connected to ground which got rid of most of the shock escape. The last two electrodes were connected to a 5-stage, capacity-coupled amplifier. When the muscle action-potentials were also studied, an insulated wire with bared tip was inserted into the belly of the muscle and connected to the proximal pick-up electrode on the nerve. There was no confusion of the action-potential of the muscle and that of the nerve on the screen of the DuMont type cathode ray oscillograph due to conduction time and junctional delay. Sometimes two leads from the muscle were used, and then the action-potential was recorded on a Davis-Grass encephalograph.

A celluloid plate with ruled lines on it, placed over the face of the oscillograph, served to measure the heights of the action-potentials which were recorded in arbitrary units as seen. The potentials recorded by the encephalograph were measured with calipers and recorded in millimeters, as were the tracings of the mechanical response which were recorded isotonically.

Curare was given *ad libitum* until curarization was complete as was evidenced by no response to an indirect tetanus of 30 per sec. Lack of response to single shocks does not indicate complete curarization. Potassium was added to the medium (40 cc.) bathing the muscle in the form of a 5 per cent solution of potassium chloride. Sometimes the 5 per cent solution was diluted with Ringer's solution before using. The solution was added dropwise near the knee-joint. Each drop was about 0.05 cc. The whole solution was then drained off and replaced by fresh Ringer's solution. Recovery from the effects of the potassium was quite rapid when small quantities were used. With very large quantities, the muscle sometimes remained in permanent contracture.

RESULTS. *The action potential of the potentiated twitch.* Table 1 and figure 1 show that the muscle action-potential accompanying a mechanically potentiated twitch is greater than that accompanying the last twitch before the potentiating tetanus.

Table 1 reveals two other facts that are important for the understanding

of post-tetanic potentiation. First, the twitch-tension of the first twitch following the conditioning tetanus (*e.g.*, after tetani 2 and 3) may be suppressed although its accompanying action-potential is increased in comparison with the values before the tetanus. This indicates that, in spite of greater excitability of the motor end plates, the muscle fiber is

TABLE 1

The relation of the twitch-tension (T) in millimeters to the magnitude of the muscle action-potential (M) in millimeters as recorded by the electroencephalograph

Single shocks, 1 per second; conditioning tetani, 30 per second for 5 seconds.

M	T	Tetanus 1 M	T	Tetanus 2 M	T	Tetanus 3 M	T
		7.5	16.4	5.4	4.1	5.5	4.0
		7.8	16.7	7.0	10.0	6.4	7.5
		7.4	14.6	8.0	10.8	6.3	6.0
		7.4	13.9	7.6	8.9	5.6	4.9
		7.1	12.4	6.8	8.2	5.1	4.5
		6.4	11.0	5.3	6.1	4.2	3.6
		5.9	9.8	5.4	5.5	3.6	3.2
		5.3	9.5	5.2	4.7	3.5	2.5
		5.4	8.4				
6.9	8.1	5.4	7.0				
6.5	7.5						
6.9	7.8	4.9	4.9	4.0	1.7	2.9	1.5
6.4	7.3	4.9	4.8	4.0	1.7		
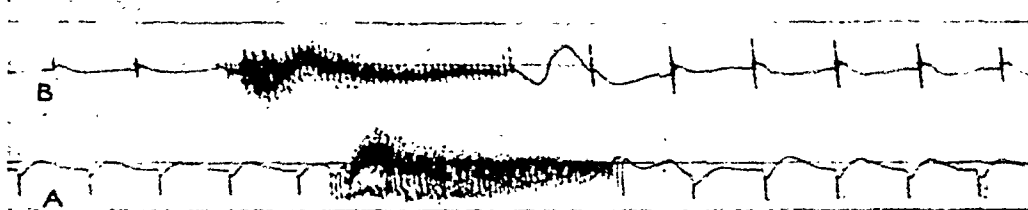
Fig. 1. Single shocks, 1 per sec.; conditioning tetani, 30 per sec. for 5 sec. Potentials recorded with encephalograph.

A. Monopolar lead, belly of muscle—distant part of bath. Untreated muscle not greatly fatigued.

B. Bipolar leads, belly-tendon. Muscle had previously been treated with potassium, but the solution had been drained and its effect had passed off 5 min. before.

unable for a short time mechanically to respond. And second, after a tetanus the magnitude of the first muscle action-potential is regularly less than the magnitude of the second. A simultaneous examination of the accompanying nerve action-potentials shows that after the conditioning tetanus the number of nerve fibers responding to the single shocks is

reduced; this accounts for the difference between the first two muscle action-potentials.

Suppression. If the conditioning tetanus is of relatively high frequency and long duration, or if the strength of the test-shock is relatively low, so few nerve fibers will respond to the test-shock that the electrical and mechanical response is less than before the conditioning tetanus or it may disappear entirely. This is called post-tetanic suppression or junctional inhibition and it is sometimes attributed to the depressant effect of a chemical mediator which has accumulated in large quantities (*e.g.,* 5, 6). However, as in figure 2, every case of "suppression" except when the muscle is completely fatigued (as shown by absence of response to a direct stimulation or failure to recover with rest)
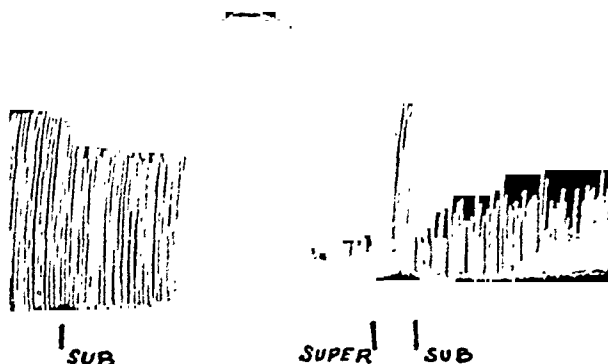
Fig. 2. Fresh, untreated preparation. Single shocks, 1 per sec.; conditioning tetanus, 30 per sec. for 30 sec. At *SUB*, shocks were made submaximal. After the tetanus the mechanical response was reduced 85 per cent, and the nerve action-potentials 70 per cent. At *SUPER*, the shocks were made supramaximal, the mechanical response and nerve action-potential becoming normal. At *SUB*, the shocks were made submaximal again. The mechanical response increased with the increase in the nerve action-potential.

can be overcome by raising the strength of the testing shock delivered to the nerve until the oscillograph shows that as many nerve fibers are responding as did before the conditioning tetanus.

A necessary condition for obtaining post-tetanic potentiation is that the testing shocks must be strongly supramaximal, for if the shocks are relatively weak and the conditioning tetani fairly fast and long, variations, up to and including extinction, appear in the picture of potentiation. In each case, oscillographic examination of the nerve fibers showed that this was due to the failure of some or all of the nerve fibers to respond on account of the raised threshold accompanying the period of positive after-potential which follows the tetanus (8). This is undoubtedly also the explanation of results obtained by Guttman *et al.* (1) and Brown and v.

Euler (2); the latter authors merely say that it is "an additional factor" coming into play.

The effect of curare on potentiation. Brown (2) claims that it is possible to obtain potentiation of the same order of magnitude in the curarized preparation as in the normal. Guttman *et al.* (1), on the other hand, reported that they could not. A confirmation of one or the other of these reports is of paramount importance in deciding whether post-tetanic
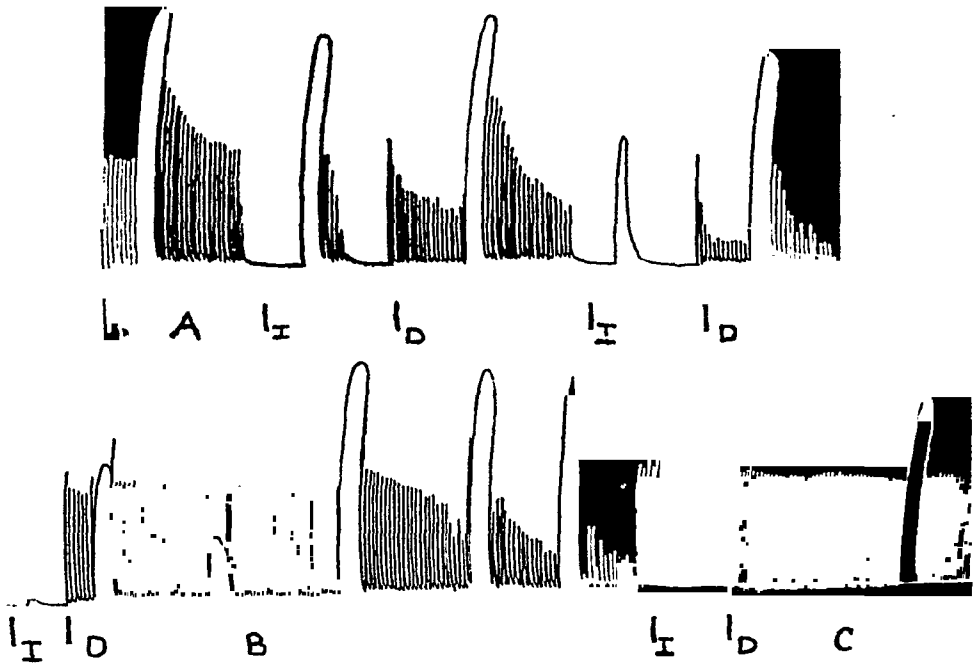
Fig. 3. Curare administered before *A*. Until the beginning of *A*, the same amount of potentiation was obtained by indirect stimulation as by direct. Single shocks, 1 per sec.; conditioning tetani, 30 per sec. for 5 sec.

A. D stands for direct stimulation, and *I* for indirect stimulation. *B.* For the third and subsequent direct tetani the voltage was doubled during the tetani. *C.* Between *B* and *C* there was a minute rest. The voltage was raised for the direct tetanus.

potentiation is a function of the myoneural junction or of the the muscle fiber.

When an indirect tetanus of 30 per sec. for 5 sec. still produces a mechanical response of the muscle, figure 3A, that is, when it has a decurarizing action, it is possible to obtain post-tetanic potentiation by direct stimulation, and this potentiation is of a magnitude comparable with that obtained by indirect stimulation. Another point to notice in figure 3A is that although the muscle responded to indirect tetani it did not respond to single shocks before the tetanus; it did afterwards due to the

decurarizing effect of the tetanus. This direct potentiation was undoubtedly due to the effect of the direct stimuli on the nerve endings or motor end-plates of the muscle, for when, as in figure 3B, there was finally no decurarizing action of a supramaximal indirect tetanus, no potentiation was obtained with direct stimulation.

In some cases it was possible to obtain an augmentation of the response to single direct shocks after a tetanus but only under certain conditions such as raising the voltage during the conditioning tetanus or rest. However, this augmentation did not always appear even when these conditions were fulfilled, *cf.* figure 3C where the preparation was both rested and the voltage of the tetanus raised with negative results. When the augmentation did appear, its magnitude never exceeded 25 per cent and it rarely lasted for more than 10 sec. This is very much below the normal range
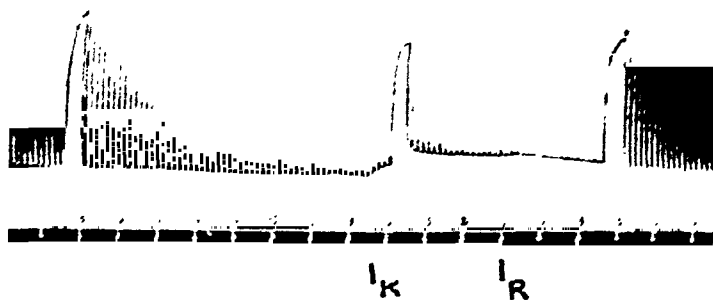
Fig. 4. Fatigued preparation, previously untreated. Single shocks, 1 per sec.: conditioning tetani, 30 per sec. for 5 sec. At K, the concentration of potassium in the bath (50 cc.) was raised 50 times. At R, this solution was drained and replaced by fresh Ringer's solution.

of increase in tension (500–1000 per cent) and of the persistence of the increase (20–60 sec.) obtained in untreated preparations by stimulation of the nerve.

If excitability of the motor end-plates was depressed by fatigue instead of by curare, the very same results were obtained with direct stimulation, namely, at any one time the magnitude of potentiation was the same for both direct and indirect stimulation, and when the myoneural junctions failed to respond to indirect stimulation, no potentiation except under the conditions described above was obtained with direct stimulation. The only difference between fatigue and curare was that the effect appeared more quickly with curare.

The effect of potassium on post-tetanic potentiation. Figure 4 shows that when the concentration of potassium was raised 50 times in the medium bathing the muscle, potentiation could still be obtained after a condi-

tioning tetanus, although this potentiation was less in magnitude and duration than those before and after the administration of the potassium. By adding the potassium slowly, only a slight contracture was produced which did not obscure the loss of irritability. When added in this way, the potassium had a curariform effect, but nevertheless, as figure 4 shows, the tetanus still had a slight decurarizing effect.

When potassium was added suddenly only two effects, if any, on potentiation, irrespective of the amount, were ever found: first, when moderate amounts were given there was a decrease in magnitude and duration of the potentiation. And second, if contracture was produced, as it was when large amounts of potassium were given, the potentiated twitches rose above the level of the contracture in such a way that from the point of view of the *crests* of the twitches, an ordinary potentiation picture was
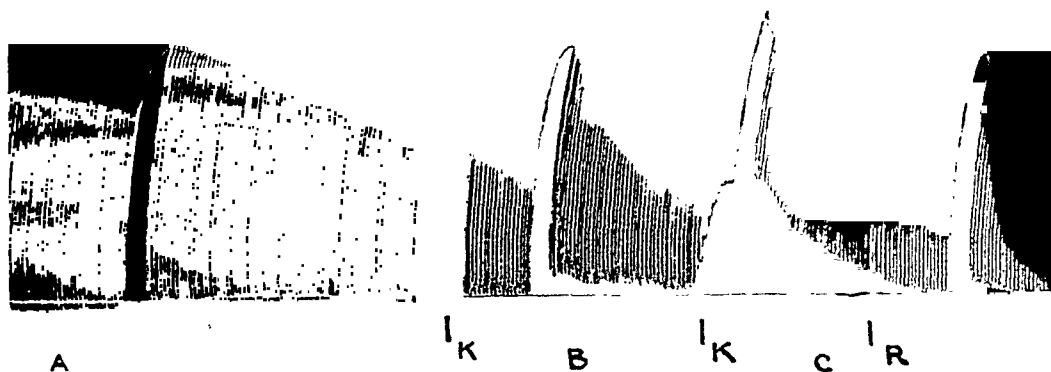
Fig. 5. Fresh, untreated preparation. Single shocks, 1 per sec.; conditioning tetani, 30 per sec. for 5 sec.

A. Normal potentiation in slightly fatigued preparation. *B.* Between *A* and *B*, 0.1 cc. of a 5 per cent solution of KCl was added to the bath (50 cc.). Potentiation picture is typical of middle fatigue. *C.* At *K*, 1.0 cc. of a 5 per cent solution of KCl was added. At *R*, this was drained off and replaced by fresh Ringer's solution.

obtained, figure 5B and 5C, although when the *absolute height* of the twitch above the contracture level was measured, there was no potentiation.

All the experiments that we have done show either a slight decrease in magnitude and duration of potentiation, or none as in figure 5B, or a contracture when potassium is added, but there was never any enhancement of the magnitude or duration of potentiation on the addition of potassium. Brown and v. Euler (2) also found that potentiation was never affected when potassium was injected into the artery during the conditioning tetanus (intact cat preparation).

Table 2 shows the effect of potassium on the twitch-tension and muscle action-potential. The twitch-tension fell gradually until a suppression of 17 per cent developed; about the 70th twitch the tension was again normal. On the other hand, the muscle action-potential fell to about 75

per cent of its original value, and this fall bore no relation to the decrease and subsequent increase in tension. With smaller doses potentiation of the twitches instead of suppression was obtained, confirming Wilson and Wright (12).

DISCUSSION. If a muscle is partially fatigued by indirect stimulation with single maximal volleys or artificially fatigued by partial curarization, a rise in threshold of some of the motor end-plates of the muscle fibers results so that these no longer respond to single volleys. In axons Erlanger and Blair (19) have termed an analogous appearance of subnormal ex-

TABLE 2

The effect of potassium on the twitch-tension (T) in millimeters and action-potential of the muscle (M) in millimeters as recorded by the electroencephalograph

Rate of stimulation, 1 per second; 0.5 cc. of a 5 per cent solution of KCl was added.

M	T	REMARKS
8.5	40.3	
8.3	39.5	
8.2	43.1	Potassium added
6.9	39.5	
6.8	37.5	
5.4	37.0	
4.5	34.3	
4.1	35.0	
3.9	34.3	
3.7	33.7	
3.5	33.2	
2.5	33.4	
2.4	35.0	Potassium drained
70 unmeasured twitches		Fresh Ringer's solution added
1.9	39.5	
1.9	35.8	
1.7	40.0	

citability "disbandment." There is reason to suppose that the two phenomena are similar, because if a succession of nervous impulses arrives at these disbanded end-plates closely enough together, as during a tetanus, the muscle fiber will finally contract. The reason for this is that each blocked impulse raises the excitability of the end-plate until one impulse finally becomes effective. In axons this process of facilitation by a train of subthreshold stimuli is called "recruitment," and it was demonstrated at an anode block by Erlanger and Blair (19) and at the stimulating electrodes during a period of after-positivity with accompanying subnormal excitability by Gasser (20). Evidence is as yet lacking to show that the

motor end-plate is in the same physico-chemical state when fatigued as is the axon during the conditions just mentioned.

After a disbanded muscle fiber has been recruited in this manner during a tetanus, its enhanced excitability may persist for from 20 sec. to 1 min. after the end of the tetanus—in frog muscle at least. Now although this phenomenon has no analogy in axons, it does have one in autonomic synapses. Larrabee and Bronk (11) showed that in the stellate ganglion the degree and duration of facilitation is increased by repetitive stimulation; for example, a short tetanus increased the response to a submaximal volley 4 to 5 times, and this enhanced excitability persisted for about 60 sec.

Thus potentiation involves recruitment of muscle fibers from a reservoir of idle muscle fibers set up by fatigue of the end-plates or, artificially, by partial curarization; this recruitment occurs during tetanic stimulation. Potentiation also involves the persistence of the enhanced excitability of the end-plates for a relatively long period after potentiation is over.

This view is consistent with the fact that potentiation cannot be obtained by direct stimulation when the muscle is completely curarized (*cf.* also 1). That is, potentiation is a myoneural process, not a function of the muscle fiber itself, as Brown (2) claims. His results were probably due to incomplete curarization as he did not use tetani to test for curarization (*cf.* 3). It is also consistent with the fact that the results of alternate direct and indirect stimulation during normal fatiguing of the myoneural junction were identical with those obtained with curare. This indicates that curarization is an artificial "fatiguing" of the myoneural junction (*cf.* 10) and also that post-tetanic potentiation is the identical phenomenon in both circumstances. Potentiation, it is known (1), cannot be obtained in the normal frog muscle when unfatigued; it can be obtained in the fresh fowl muscle where some end-plates normally never respond to a maximal single volley. Brown (4) says that potentiation in the latter case is like that of the partially curarized cat muscle where the increased tension is due to an increased number of fibers responding to the nerve volley.

Still further support is found in the fact that in the frog at least the muscle action-potential accompanying the potentiated twitch is increased while that of the nerve is decreased when compared with those accompanying the last twitch before the conditioning tetanus. This means that, although fewer motor units respond to the testing shock after the tetanus due to the raised threshold of the nerve fibers which accompanies the positive after-potential produced by the tetanus (8), more muscle fibers are excited to contract within the motor units that do respond, thus producing an increased twitch tension.

This is confirmed by the direct findings of Asmussen (9) who found that not all the muscle fibers in a motor unit respond to a single volley;

during a tetanus, however, the number of active end-plates increased steadily until fatigue set in. The fatigue may be end-plate fatigue (the "all-or-none" response) or fiber fatigue (the "graded" response); in the isolated nerve-muscle preparation he observed that end-plate fatigue intervened first.

Chemical versus electrical theory of potentiation. Chemical. Because of the rapidly destructive action of choline esterase, acetylcholine cannot be the cause of relatively long-enduring potentiation (2). Instead a mobilization of potassium at the myoneural junction is believed to cause potentiation because the effects of injected potassium on single twitches mimic the effects of tetani (2, 3, 5). There are several facts, however, that militate against this. Fenn (13) concluded that either no potassium left cat nerve fibers during tetanization or else that it returned so rapidly that his methods of analysis were unable to detect its escape; the tetani he used were much faster and longer than ordinary potentiating tetani. We found, and Brown (2) did also, that no combination of tetanus and administered potassium had any effect on the duration and magnitude of post-tetanic potentiation. An excess of potassium suppresses the mechanical response of single twitches; a prolonged, high frequency tetanus, however, exerts its effect on the nerve and not at the myoneural junction (*cf.* fig. 2). In large doses potassium seems to have a curariform effect, yet a conditioning tetanus still evokes potentiation (*cf.* fig. 4). If potassium were mobilized at the myoneural junction during the tetanus, a still further depression of excitability would be expected. Finally, we agree with Brown's (15) conclusion that injected potassium exerts its augmenting effect on single twitches by acting on the contractile mechanism of the muscle fiber ("graded" response) and not at the myoneural junction, the site of the potentiating effect of a tetanus.

Electrical. Erlanger (16, 19) points out the similarity of the time-course of changes in excitability after a subthreshold stimulus at the muscle synapse (*cf.* 10), at a block in the axon, at the stimulating electrodes on axons, and at the synapses of motoneurons. This leads him to believe that "the common denominator obviously is electrical," that at an artificial synapse in an axon, and also by inference, at a natural synapse, the action-potential spike acts as an electrical stimulus to the tissue beyond (19), and that the chemical theory of transmission does not explain the facts (16).

That this is the explanation of the recruitment phase of potentiation is supported by the fact that the times for the latent addition (10) of two subthreshold impulses at the frog muscle synapse (optimum, 4 msec.; maximum, 70 msec.) fit fairly well with potentiating frequencies, for example, the lower limiting frequency for the frog is around 10 per sec. (1). The optimal frequency, however, is given as 30 per sec.; this dis-

crepancy is due to the fact that higher frequencies produce a subnormal excitability in the nerve, which prevents the appearance of potentiation (*cf.* fig. 2). With strong test shocks, however, we have observed potentiation with tetani of 100 per sec., and Feng *et al.* (5) reported it with tetani of 240 per sec. Katz (14) showed that a subthreshold cathodal current to the region of the myoneural junction could facilitate a blocked nervous impulse and that the facilitation curve was the same as that for latent addition. This facilitation is obtainable *"after the withdrawal* of a direct electrical stimulus," and, therefore, in his opinion, it is due to the subsidence of the local excitatory effect of the stimulus and not of a transmitter substance. The rate of subsidence of the local excitatory effect which outlasts the nervous impulse is characteristic of the alpha excitability of muscle (21). The local excitatory effect can be observed at the myoneural junction as it produces electrical changes whose time-course differs from those associated with the propagated disturbance (17). There is no evidence that this local effect is due either to acetylcholine (18) or to potassium (15). Nor is there any evidence that the prolonged enhanced excitability lasting as long as 1 min. following the tetanus is due to a persistence of potassium at the myoneural junction.

The question of Bronk and Brink, quoted at the beginning of this paper, may be answered by saying that potentiation in the normal sympathetic ganglion resembles potentiation in the normal muscle as well as the partially curarized, and that, further, it seems likely that the mechanism is the same in both.

SUMMARY

The problem of post-tetanic potentiations was studied in the isolated frog sciatic-gastrocnemius preparation. The muscle and nerve action-potentials and the twitch-tension were observed simultaneously. Particular attention was paid to the effect of curare and potassium on potentiation.

Potentiation was not obtained in the completely curarized preparation by direct stimulation. It was obtained in the partly curarized preparation. Normal fatiguing of the myoneural junction gave the same results as curare did.

Potentiation was obtained after the administration of depressing doses of potassium. Potassium never enhanced the potentiating effect of tetani.

The muscle action-potential accompanying the potentiated twitch was greater than that accompanying the twitch preceding the conditioning tetanus. The nerve action-potential after the tetanus was usually decreased.

Suppression of the muscle response after a prolonged tetanus was due to a decrease in the number of nerve fibers responding to the test-shock.

This suppression could be overcome by raising the strength of the test shock.

These results were discussed in the light of current explanations of potentiation which were found to be inadequate. It was concluded that potentiation was the same in the partly fatigued frog, partially curarized mammal, and normal fowl muscle and that it was due to the facilitating effect of blocked nerve impulses and an enhanced local excitatory state persisting at the motor end-plates after tetanization. It was suggested that since others have shown the inadequacy of the chemical theory of neuromuscular transmission in explaining these events, a chemical explanation of post-tetanic potentiation is also inadequate.

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ANOXIC EFFECTS OF HIGH OXYGEN PRESSURE ON SMOOTH MUSCLE[1, 2]

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Exposure of animals to oxygen at high pressure induces changes which involve smooth muscle tissue. The reaction in this tissue might conceivably arise as a result of a disturbed carbon dioxide transportation by the blood or as a result of a more direct effect of high oxygen on tissue. In either case the exact mode of action could be one or several of a possible great variety. The experiments herein reported were undertaken then with a number of questions in mind: First, are the effects of high oxygen on smooth muscle necessarily dependent on central nervous or hematogenous connections? Second, if these effects can result from a peripheral influence of high oxygen is such influence mediated through intrinsic nerve endings, or are they due to a "direct" action on the effector itself? Third, what is the nature of this direct action?

The smooth muscle investigated was that obtained from the gastrointestinal tract of a rabbit freshly killed by a postcephalic blow by hand. The gut was removed, its lumen gently rinsed, and suspended in a bath of Tyrode solution within a pressure chamber. This bath was continuously bubbled with pure oxygen saturated with water vapor and warmed to 37.5°C. An isotonic optical lever was used to record the rhythmic contractions and changes in tonus. Longitudinal duodenal and pyloric sphincter tissues were studied.

RESULTS AND DISCUSSION. *Duodenum.* Exposure of longitudinal duodenal muscle to oxygen at 75 pounds' pressure resulted in a progressive decrease in tonus. This decrease became evident in some experiments within 20 minutes and invariably was very pronounced in all, one hour after cessation of compression. The regular rhythmic contractions, which at atmospheric pressure recurred at a frequency of 14 per minute, were replaced by contractions of irregular amplitude. Frequently, and especially so in the longer exposures, the amplitude of the rhythmic contractions diminished so that for short intervals there was complete cessation of rhythmic contractions. Not only was the amplitude diminished by high

oxygen but also the frequency was decreased. In the more prolonged exposures there appeared a gradual development of recurrent spasmodic changes of tonus. These recurrent tonic changes interspersed by periods of relative quiescence increased in severity as the pressure was maintained and continued on into the early stages of decompression.

Decompression to atmospheric pressure was invariably followed by a reversal of these compression effects. The long spasmodic waves in tonus gradually gave way to a steady tonus level which in many instances was appreciably higher than that obtaining preceding compression; the frequency of rhythmic contractions returned to normal; the amplitude became regular, but with few exceptions was smaller than that preceding compression. A second exposure of the tissue to high oxygen was attended by an earlier onset of effects essentially similar to those recorded in the first exposure; the recovery on the second decompression, however,
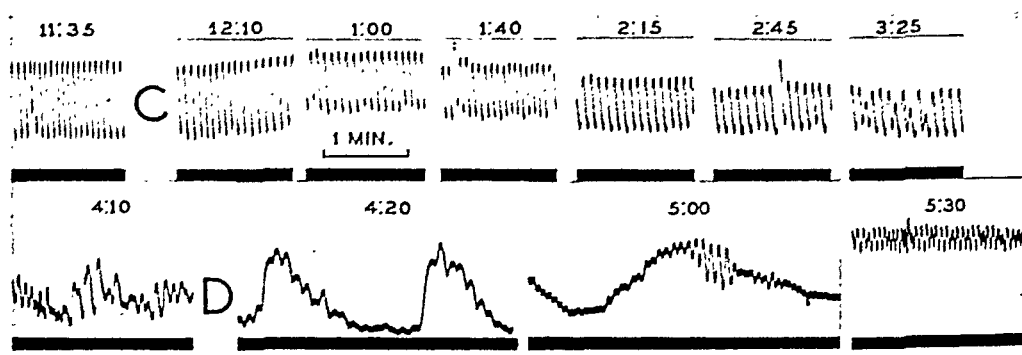
Fig. 1. The effects of oxygen at high pressure on a longitudinal duodenal strip (rabbit) in Tyrode solution. Time of day is indicated above each recording. *C* indicates compression of oxygen to 75 pounds' pressure; *D* indicates point of decompression to atmospheric pressure.

was less complete than that following the first exposure. Typical results derived from an experiment in which a longitudinal duodenal strip was employed are shown in figure 1.

The tonus changes which occurred in a control duodenal strip exposed to oxygen at atmospheric pressure and those induced in a similar preparation by exposure to high oxygen pressure are contrasted in figure 2, part 1. The earlier onset of effects in successive exposures as well as the slower and less complete recovery on decompression from successive exposures are well illustrated in this graph. The overshooting of the precompression tonus level on decompression (see fig. 1) appears from this graph to be a rapid resumption of the progressively increasing tonus obtaining before the first compression. This interpretation is supported by the practical coincidence of the control curve with that of the test. The changes in amplitude of the rhythmic contractions of these same control and test preparations,

the tonus changes of which are shown in part 1, are plotted in part 2 of figure 2.

These results clearly indicate that oxygen at high pressure can profoundly affect isolated smooth muscle and that the reaction of this tissue in animals exposed to high oxygen pressure is not necessarily dependent upon nervous connections to the central nervous system, or upon hematogenous connections to remote sources of humoral substances such as adrenalin, or
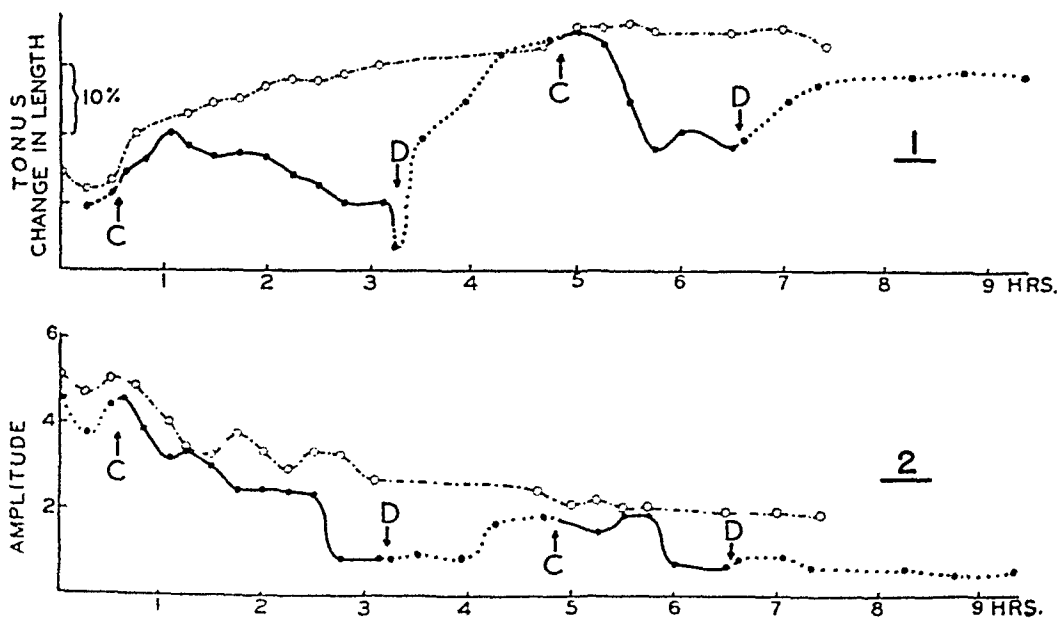
Fig. 2. *Part 1.* In the upper curve (dot and dash) the change in tonus (relaxed length) of a strip of rabbit duodenum exposed to oxygen at atmospheric pressure in Tyrode solution is plotted against time in hours on the abscissa.

In the lower curve the changes in tonus induced in a similar preparation (from the same animal) by exposure to oxygen at 75 pounds' pressure. The dotted line represents tonus during exposure to oxygen at atmospheric pressure; the solid line represents tonus during exposure to oxygen at 75 pounds' pressure. C and D indicate the points of compression and decompression respectively; C'' and D' the same for a second such exposure.

Part 2. These curves show the changes in amplitude of the rhythmic contractions of the preparations whose tonus changes are shown in part 1.

on an upset in the carriage of carbon dioxide by the blood. It is recognized, however, that in an animal poisoned by high oxygen, the mediation of some effects over nervous and hematogenous connections to peripheral structures does occur as a result of the hypercapnia which arises from a disturbed function of hemoglobin in carbon dioxide transportation.

It is conceivable that the response of isolated smooth muscle to oxygen at high pressure might be due to some action of oxygen on intrinsic nerve plexuses, nerve endings or the effector cells themselves. The precise

evaluation of the possible contributions by these structures to the final response is a difficult problem especially if "there is no drug which has on plain muscle an action clearly comparable with that of curare on striated muscle in paralyzing the nerve endings" (Evans, 1926). However that may be, if intrinsic nerve plexuses and endings do play an intermediate rôle in the production of the high oxygen effects we should expect that such effects would simulate those elicited by neurohumoral substances such as adrenalin and acetylcholine. Experiments were therefore performed in which various dilutions of adrenalin and of acetylcholine were added to the Tyrode baths of fresh duodenal tissue. In each instance the response of the muscle to either of these substances was so very dissimilar from the high oxygen response as to indicate that if the intrinsic nerves or their endings are affected by high oxygen they do not mediate any effects via these neurohumoral substances.

Pyloric sphincter. The possibility that the endings of the sympathetic and parasympathetic supply to the pylorus may not always have motor and inhibitory functions identical with the corresponding endings in the duodenum, suggested that further information concerning the possible involvement of intrinsic nerves in oxygen poisoning of isolated tissue might be uncovered by a study of the effects of oxygen at high pressure on pyloric sphincter muscle. Experiments were therefore carried out in which the procedure was essentially the same as that employed in the experiments on the duodenal tissue. It was found that raising the oxygen pressure from atmospheric to 75 pounds induced a fall of tonus and that decompression to atmospheric pressure was followed by a return of tonus to the precompression level, effects similar to those recorded from duodenal strips. Typical results of these pyloric sphincter experiments are shown in figure 3 (I, II, III) where tonus (relaxed length) is plotted against time in minutes. Similar treatment with air failed to alter the tonus and so rules out the possibility that the effects produced by oxygen at high pressures are due to pressure alone. A subsequent exposure of the same tissue to oxygen at 75 pounds' pressure gave effects (graph III) essentially similar to those induced by the first exposure to high oxygen. If then it be assumed that the functions of nerve endings in the pylorus are not identical with those in the duodenum, the similarity of action of oxygen at high pressure on these two tissues would indicate a non-involvement of the intrinsic endings.

If we assume that the parasympathetic endings are inhibitory to the pyloric sphincter and that this inhibitory influence is accomplished through a liberation of acetylcholine the question arises: Does the decrease in tonus of pyloric sphincter muscle which occurs during its exposure to high oxygen pressure depend upon a stimulation of parasympathetic nerve endings and a release of acetylcholine? So in another attempt to answer this question of intrinsic nerve ending involvement in oxygen poisoning, atropine was

used. Now although atropine might not be expected to completely paralyze parasympathetic action (due perhaps to intracellular nerve endings, Cannon and Rosenblueth, 1937) one may justifiably expect that it would at least diminish extracellular parasympathetic influence. Several experiments were therefore performed in which the Tyrode bath of sphincter preparations was atropinized previous to their exposure to high oxygen pressure. Such treatment did not alter the effectiveness of high oxygen in decreasing the tonus. Similar atropinization of longitudinal duodenal strips likewise did not alter the response of this tissue to high oxygen
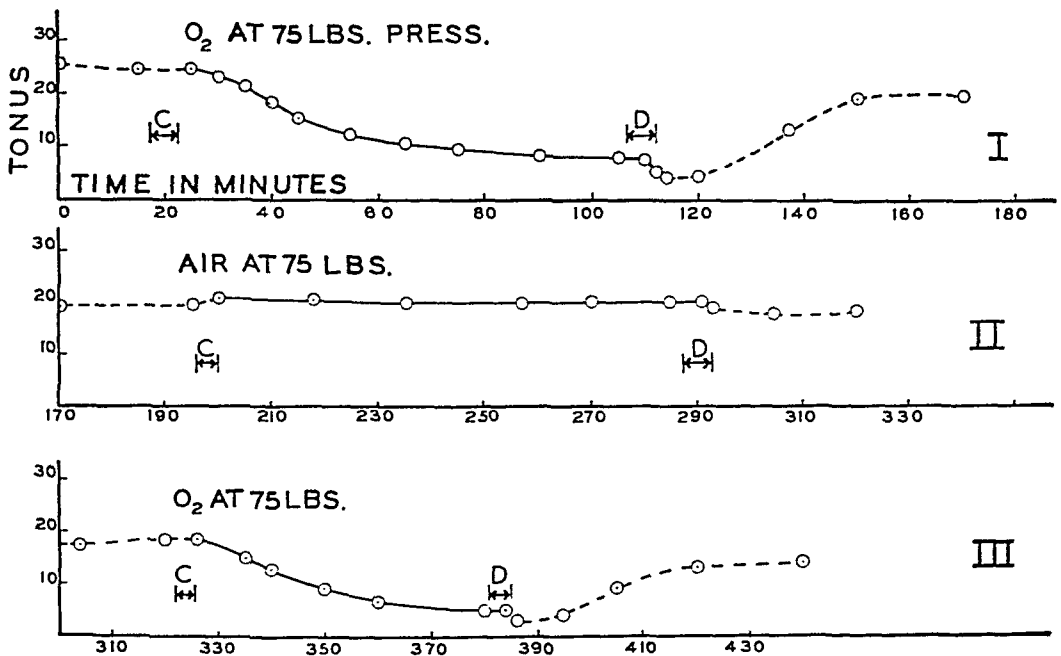
Fig. 3. Pyloric sphincter muscle. I. Tonus change as elicited by oxygen at 75 pounds' pressure. II. The tonus of the same tissue exposed to air at 75 pounds' pressure. III. Tonus change in the same tissue as a result of a second exposure to oxygen at 75 pounds' pressure. The duration of exposure to increased pressure of 75 pounds is indicated by the solid line portion of each curve. The broken line indicates exposure to oxygen (or air II) at atmospheric pressure. C and D indicate compression and decompression respectively.

pressure. While the results of these atropinization experiments do not offer conclusive evidence they do lend support to the belief that parasympathetic endings or parasympathomimetic substances are not essential intermediaries in the oxygen poisoning of isolated smooth muscle.

Cyanide, low oxygen. In oxygen poisoning of the circulated animal there occurs a decrease in oxygen consumption (Bert, 1878; Hill and Macleod, 1903; Bean, 1931). This is due in part to an increased acidity arising from a disturbed carbon dioxide transportation but apparently there is a second factor contributing to the lowered oxygen consumption, *viz.*, a poisoning

of respiratory enzymes by the high oxygen pressure. One would expect
.this enzyme poisoning to be operative also in isolated tissues exposed to
high oxygen and the question arises of whether the lowering of oxygen
consumption consequent upon such enzyme poisoning might not play a
very significant rôle in the induction of those reactions seen in the tissue
exposed to oxygen at high barometric pressure. In light of the possibility
that the effects of oxygen poisoning might be attributed, in part at least,
to the lowered oxygen utilization, a comparison of the effects of high
oxygen on these tissues, with those effects produced by cyanide and by
low oxygen, is of especial interest.
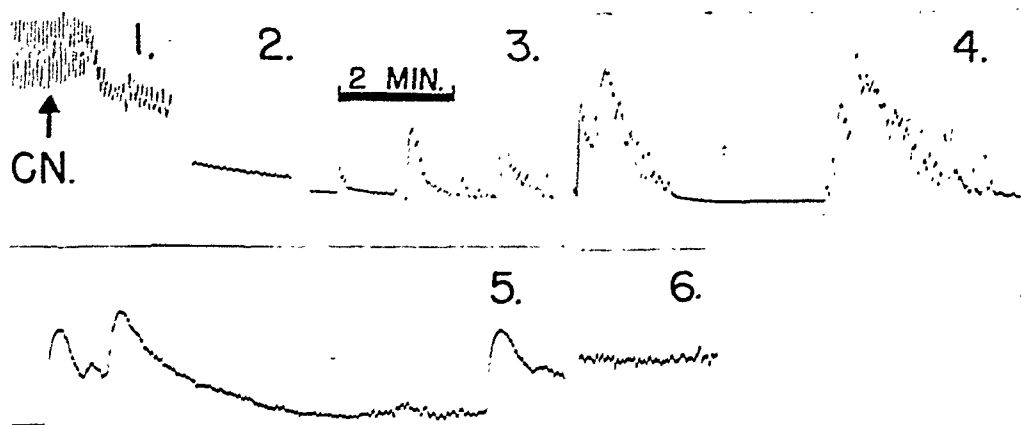
Fig. 4. The effects of sodium cyanide in high concentration on isolated longi-
tudinal duodenal muscle of the rabbit: part 1, administration of cyanide (CN.)
to give ultimate concentration of 1:5000 in Tyrode solution bubbled with oxygen
at atmospheric pressure; part 2, 7 minutes after administration; part 3, 15 minutes
after administration; part 4, 30 minutes after administration and beginning of com-
pression of oxygen; part 5, after 50 minutes' exposure to oxygen at 75 pounds' pres-
sure; part 6, 2 hours after decompression to atmospheric pressure.

To investigate the effects of cyanide on the smooth muscle under our
experimental conditions, sodium cyanide was added to the oxygenated
Tyrode bath in amounts sufficient to give an ultimate concentration of
1:1,000,000 or greater. Such procedures induced a fall in tonus and a
decrease in frequency and amplitude of the rhythmical contractions of
longitudinal duodenal tissue. In concentrations greater than 1:60,000
there occurred prolonged periods during which the rhythmical contractions
were absent, interspersed by spasmodic waves of increased but unsustained
tonus—effects which were essentially similar to those recorded in the more
prolonged exposure to high oxygen pressure. The characteristic action
of sodium cyanide in the higher concentrations on the longitudinal duodenal
strip is shown in figure 4. The low tonus, the relative quiescence and ir-
regularity of the rhythmic contractions interspersed by recurrent spas-

modic waves of increased tonus are features typical of the response of duodenal longitudinal muscle to oxygen at high pressure.

It is generally agreed that the action of cyanide on living tissue is due to the lowered oxygen utilization. Nevertheless a study of the effects of administering low percentages of oxygen on these tissues at atmospheric pressure is of interest here because unlike the cyanide effects, the responses induced by low oxygen administration are not dependent upon the poisoning of respiratory enzymes. A gaseous mixture of low oxygen (5 per cent) and nitrogen (95 per cent) previously warmed and saturated with water vapor was bubbled at atmospheric pressure into the Tyrode bath of a fresh strip of duodenal tissue. This gaseous mixture gave no significant alteration either in tonus or in the rhythmic contractions of the tissue. Gas mixtures of 3 per cent oxygen and 97 per cent nitrogen, however, induced a response which duplicated that induced by cyanide or high oxygen pressure. The administration of pure nitrogen brought about a more rapid onset of the same response and magnified the characteristic features. These results not only provide a comparison of low oxygen percentage effects with those of cyanide and high oxygen pressure but they also emphasize the wide margin of safety—so far as oxygen supply is concerned—within which the intestinal muscle *in vivo* functions under normal conditions.

Hyperoxic anoxia. The results presented thus far bring us to the belief that, while enzyme poisoning by high oxygen could conceivably lead by devious routes to a variety of changes in the tissue, all of which might contribute to the response of smooth muscle described above, the arresting similarity between the effects on isolated smooth muscle, of sodium cyanide, of low percentage of oxygen at atmospheric pressure and of oxygen at high barometric pressure is based on some underlying factor common to all three conditions and it appears this common factor is an anaerobic metabolism. In oxygen poisoning it would seem we have a paradoxical relationship in which a superabundant supply of oxygen gives rise to effects induced by oxygen want—a relationship which may perhaps be best described as a hyperoxic anoxia. In view of such probability it is not surprising that periodic breathing—a well known response to low oxygen administration—frequently occurs also in oxygen poisoning (Bean, 1932). Moreover, the finding of an increase in blood lactic acid in anesthetized animals breathing oxygen at high pressure lends further credence to the hyperoxic anoxia interpretation of acute oxygen poisoning.

In a series of seven of our present experiments the lactic acid content of the Tyrode bath exposed to oxygen at 75 pounds' pressure and in which the smooth muscle (duodenum) had shown some oxygen poisoning reaction, was determined. In six of these the lactic acid content was less than that found in the controls. This would perhaps seem to bring into ques-

tion the significance of the anaerobic metabolism as a factor in oxygen poisoning in these isolated smooth muscle preparations. It should be noted, however, that the oxidation of lactic acid intracellularly in intimate relationship with tissue fluids, or in the blood may be quite different from its oxidation in Tyrode solution under high oxygen pressure.

Even though it be granted that the parallelism in the responses of these isolated smooth muscle preparations to sodium cyanide and to oxygen at high pressure can be explained on the basis of a common anaerobic metabolism it must be recognized that there may be points of divergence in the toxic action of these two conditions. This is suggested by the finding that the effects of sodium cyanide and of high oxygen pressure administered in combination are not always directly and completely additive.

In order to determine whether there might be some unsuspected toxic substance released by the isolated tissue during its reaction to high oxygen pressure, the Tyrode bath was drawn off at the height of the muscle's response to high oxygen. This was then used as a bath for a normal piece of rhythmically contracting duodenum but the tonus and rhythm remained unaltered. If there is any toxic substance produced in the isolated smooth muscle during exposure to high oxygen, either it does not diffuse into the bath in significant amounts, or it is rapidly dissipated by high oxygen in the Tyrode solution or by decompression to atmospheric pressure.

SUMMARY

Exposure of isolated longitudinal duodenal muscle of the rabbit to oxygen at 75 pounds' pressure resulted in a progressive decrease in tonus, a decrease and irregularity in amplitude of the spontaneous rhythmic contractions and a decreased frequency of this rhythm to a point of periodic cessation interspersed by spasmodic unsustained increases in tonus. Decompression to atmospheric pressure reversed these effects.

Exposure of pyloric sphincter (rabbit) to high oxygen pressure likewise resulted in a decrease in tonus which was reversed by decompression.

The effects of high oxygen on intestinal smooth muscle in circulated animals are not necessarily dependent upon central nervous or hematogenous connections but may be explained as due to a peripheral influence of oxygen at high pressures.

Atropinization of duodenal smooth muscle preceding compression failed to alter the action of high oxygen pressure on this tissue. If the intrinsic nerve endings are affected by high oxygen it would appear that they do not mediate any effect via physiological sympathomimetic or parasympathomimetic substances.

The results indicate that the peripheral influence of oxygen at high pressure is due to a direct action on the effector cells themselves.

The finding that the effects of oxygen at high barometric pressure on isolated smooth muscle are so very similar to those effects induced not only by sodium cyanide but also by the administration of low percentages of oxygen at atmospheric pressure, is interpreted as indicative of a common causal factor operating in all three conditions.

Evidence is presented which supports the suggestion that this common factor is an increased intracellular acidity which in the case of the direct action of oxygen at high barometric pressure may arise from the diminished oxygen utilization consequent upon a poisoning of respiratory enzymes. Reasons are cited for believing that this interpretation remains valid despite the failure to demonstrate any increase in the lactic acid content of the Tyrode solution bathing the tissue exposed to high oxygen pressure.

The seemingly paradoxical relationship in acute oxygen poisoning where a superabundance of oxygen at high barometric pressure elicits responses typical of those induced by an oxygen deficiency and anaerobic metabolism is referred to as hyperoxic anoxia.

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OBSERVATIONS ON THE BEHAVIOR OF DIODRAST IN THE DOG[1]

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If a substance is completely removed from the blood on one renal passage, its blood clearance is a measure of renal blood flow (RBF); if it is completely removed from the plasma, while it is absent from or fixed in the blood cells during such passage, its plasma clearance is a measure of renal plasma flow (RPF). Regardless of the degree of completeness of renal extraction and regardless of distribution between cells and plasma, RBF is measured by the expression blood clearance/blood extraction, where blood extraction is .

$$\frac{\text{arterial blood level} - \text{renal vein blood level}}{\text{arterial blood level}}.$$

If a substance is incompletely extracted from the plasma and is absent from or fixed in the cells, its plasma clearance/plasma extraction is a measure of RPF. If it is incompletely extracted from the plasma and some of it moves from cells into plasma during a renal passage, its plasma clearance/plasma extraction is higher than RPF. It is assumed that from the curve of momentary (observed) extractions can be plotted the mean extractions for the respective urine periods. The slight errors due to loss of water from blood into urine and to loss of materials through renal lymph are neglected.

Suitability of various substances for measuring RBF or RPF. Urea. Urea was used by Van Slyke, Rhoads, Hiller and Alving (5) as a reference substance for RBF determinations. It was later recognized that urea is not the most suitable substance, because of wide and transitory fluctuations in extraction (6). We confirm the following findings of Van Slyke, Hiller and Miller (7). First, that when urea is added to dog blood *in vitro* the equilibrium distribution between plasma and cells is attained very quickly (30 sec. of mixing, followed by centrifugation), with urea per 100

cc. of water being the same in cells and plasma. Second, that urea diffusion *in vivo* is rapid enough that the cell/plasma urea ratio is the same in renal vein and in arterial blood. We have carried out the further experiment of making known dilutions of blood with urea free solutions and find that the equilibrium cell/plasma urea ratio is attained as quickly when urea is diffusing out from cells *in vitro* as when it is diffusing into them. It is thus confirmed that urea plasma clearance/urea plasma extraction will be greater than RPF, because of cell contribution of urea.

Creatinine. We confirm the following findings of Van Slyke, Hiller and Miller (7). First, that when creatinine is added to dog blood *in vitro* it enters the cells very slowly; the ratio of cell to plasma creatinine rises slowly and does not reach unity even after 5 hours. This is true with beef blood (slight rouleau formation with resultant high cell-plasma interface area) as well as with dog blood (marked rouleau formation). Second, it was confirmed that diffusion equilibrium of creatinine is attained *in vivo* at between 1 and 2 hours.

In order to find whether the rate of passage of creatinine from cells into plasma is the same as that from plasma into cells, the following experiment was performed. A dog was given 0.2 gram creatinine per kilo intravenously; 1 hour after the injection $\dfrac{[Cr]_c^w}{[Cr]_p^w}$ was found to be 0.99.[2] To 20 cc. of this blood were added 4 cc. of isotonic sodium chloride. A sample was taken from this and centrifuged immediately after mixing and another sample taken 20 minutes later. The ratio was raised immediately, due to the plasma dilution, and did not fall perceptibly in the next 20 minutes. The results are seen in table 1. This experiment shows that the passage of creatinine from cells into plasma as well as from plasma into cells *in vitro* is a very slow process.

Finally, we confirm Van Slyke, Hiller and Miller (7) in finding that the creatinine content of renal vein cells is the same as that of arterial cells, although renal vein plasma is of course lower than arterial plasma. It is thus confirmed that creatinine plasma clearance/creatinine plasma extraction is a measure of RPF.

Inulin. We confirm Van Slyke, Hiller and Miller (7) in finding that there is no inulin in the blood cells of dogs after a long period of *in vivo* equilibration. We also find the same on man. Inulin plasma clearance/inulin plasma extraction is thus a measure of RPF.

Diodrast. The organic iodine compound, diodrast (D), has been pro-

[2] It may be pointed out that a ratio of unity at a given time after a single intravenous injection does not necessarily mean that this ratio will be maintained thereafter, since, with a rapidly falling blood level, plasma creatinine falls faster than cell creatinine. The mere establishment of a unity ratio is, however, sufficient for the purposes of the experiment being described here.

posed by Smith and collaborators (8) (9) (10) as essentially meeting the conditions that it is completely extracted on passing active renal tissue and that it is absent from the cells.

Diodrast in cells. The statement of Smith (8) that D is absent from dog and human cells is based upon essentially complete recoveries from plasma of D added to drawn blood. We also find very little entrance of D into the cells of drawn blood, slightly more with dog than with human. Thus, D to the concentration of 4.55 mgm. iodine (I) per 100 cc. was added to freshly drawn heparinized human blood. After 4 minutes, with mixing, centrifugation was begun and carried on for 20 minutes at 2800 rpm. Plasma showed 7.32 and cells 0.53 mgm. I per 100 cc. Since plasma fraction of hematocrit reading (V_p) was 0.428, the amount of I found by analysis and hematocrit is 4.19 + 0.227 or 4.42 mgm. per 100 cc. of blood, a fair check. Assuming that 4 per cent of the volume of the packed cells

TABLE 1

Failure of creatinine to pass from cells into plasma in vitro in 23 minutes

V_p is plasma fraction from hematocrit. Calculated whole blood creatinine was obtained from plasma and cell analyses and hematocrit.

SAM-PLE	TIME	V_p	PLASMA CREATI-NINE	CELL CREATI-NINE	WHOLE BLOOD CREATI-NINE BY ANALYSIS	WHOLE BLOOD CREATI-NINE, CALCU-LATED	CELL WATER	PLASMA WATER	$\dfrac{[Cr]_c^w}{[Cr]_p^w}$
			mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*gm. per 100 cc.*	*gm. per 100 cc.*	
3	10:25	60.2	18.6	13.8	16.8	16.6	70.66	94.44	0.99
4	10:28	66.5	13.9	13.2	13.9	13.7		95.83	1.29
5	10:48	66.7	13.8	13.2		13.7		95.83	1.29

is plasma, we find 0.25 mgm. I per 100 cc. of actual cells, about 3 per cent of the concentration in the plasma.

Diodrast to the concentration of 5 mgm. I per 100 cc. was added to heparinized dog blood and mixing accomplished by inverting every 3 seconds for 30 seconds. A sample of 5 cc. was removed at 30 seconds and its centrifugation for 20 minutes at 2800 rpm was begun 3 minutes after addition of D. The remainder of the blood stood undisturbed until 24 minutes after addition of D, when it was centrifuged. The sample obtained after 3 minutes showed 9.27 mgm. I per 100 cc. plasma and 0.88 mgm. per 100 cc. cells; assuming that 4 per cent of the packed cell volume is plasma we find 0.53 mgm. I per 100 cc. cells or 6 per cent of the plasma concentration. The sample after 24 minutes showed 8.75 mgm. I per 100 cc. plasma and 0.48 per 100 cc. cells; this gives a true cell content of 1.17 mgm. per 100 cc. or 13 per cent of the plasma concentration. Other similar experiments have given similar results.

The above findings show that D added to drawn blood passes from plasma into cells very slowly in human and somewhat more rapidly in dog blood. When, however, the equilibration is carried out *in vivo*, i.e., after and during intravenous injection of D, the rate of passage is much higher, equilibration being complete in 30 minutes or less. We give an intravenous injection of an amount of D and of inulin estimated to give the desired plasma concentration (priming infusion) and maintain this by a continuous or sustaining infusion; no sulphate or other diuretic is added. We have shown repeatedly that the plasma concentrations of creatinine, of inulin and of D are the same in simultaneous samples from an artery and from a systemic vein; the term "arterial" in this paper is applied to blood either from an artery or from a leg or jugular vein. The dogs were deprived of food but not of water since the evening before an experiment and were given 2.5 per cent body weight of water by stomach tube 45 to 60 minutes before the first urine period began. The sustaining infusion was begun 25 to 45 minutes before beginning the first urine period and immediately following the priming infusion. When blood levels were changing rapidly (after cessation of infusion) a time correction was made on the assumption that urine is formed two minutes before it enters the bladder. Urine was collected in graduate cylinders and the volume noted; the bladder was washed out with 20 cc. of saline from a syringe and the urine thus recovered noted from the increment in volume. Each complete urine sample was transferred to a 250 cc. volumetric flask and made up to volume. The excretion of the substances concerned in milligrams per minute was thus determined; from this and from the urine flow in cubic centimeters per minute the urinary concentration in mgm. per 100 cc. was calculated.

Not only the passage of D from plasma into cells but also that from cells into plasma is quite rapid, more so in the dog than in the human. This is seen when the blood level is allowed to fall; if the rate of fall is not too rapid the cell/plasma ratio remains constant. Whether the more rapid rate of passage *in vivo* means that the cell permeability is decreased *in vitro*, or that mechanical factors of the circulation are more effective in promoting equilibration than is mixing in the test tube, is not certain; perhaps both factors are operative. Furthermore, the equilibrium concentration of D in mgm. per 100 cc. of water is never as high in the cells as in the plasma. The significance of this is not clear but it apparently means that part of the water in the cell is either inaccessible to D or is incapable of dissolving D. These points are illustrated in table 2. Here the priming infusion was given rapidly (not through a catheter) and the sustaining infusion was at such a rate that the plasma D level was allowed to fall slowly for a while.

The average equilibrium value of the ratio $[D]_c^w/[D]_p^w$ for arterial blood is 0.58, with extremes 0.48 to 0.73. The average is of 33 observations

with plasma levels from 1.3 to 50 mgm. I per 100 cc.; the value of the ratio is independent of the plasma D level.

If the rate of fall of the blood D level is sufficiently high, as with cessation of infusion after a maintained high level, diffusion of D from cells

TABLE 2

Passage of D from cells into plasma in vivo to maintain constant cell/plasma ratio with slowly falling plasma level

Constancy of ratio is usually seen even where plasma level is falling much more rapidly than in this experiment. Priming infusion 12:20 to 12:22. Sustaining begun at 12:22 and continued throughout experiment.

TIME	ARTERIAL IODINE		$\dfrac{[D]_c^w}{[D]_p^w}$
	Plasma	Cells	
	mgm. per cent	*mgm. per cent*	
12:38	6.43	3.37	0.64
1:08	4.97	2.54	0.62
1:32	4.55	2.34	0.63
2:03	4.43	2.23	0.61
2:19	4.43	2.22	0.61

TABLE 3

Behavior of arterial and renal vein cell/plasma D ratios with rapidly falling plasma level

Priming infusion 10:34 to 10:44. Sustaining begun at 10:44 and stopped at 12:06. K1, 2/6/40.

TIME	ARTERIAL IODINE		ARTERIAL $\dfrac{[D]_c^w}{[D]_p^w}$	TIME	RENAL VEIN IODINE		RENAL VEIN $\dfrac{[D]_c^w}{[D]_p^w}$
	Plasma	Cells			Plasma	Cells	
	mgm. per cent	*mgm. per cent*			*mgm. per cent*	*mgm. per cent*	
11:03	29.4	11.35	0.47				
11:28	26.0	11.40	0.54	11:27	13.8	10.3	0.96
11:55	25.2	11.39	0.56	11:54	13.5	10.2	0.97
Infusion stopped at 12:06							
12:15	18.7	10.0	0.67	12:14	5.18	8.35	2.1
12:28	11.31	7.49	0.85	12:27	3.56	6.50	2.46
12:55	5.70	5.80	1.09	12:54	1.60	3.08	2.57
1:26	2.73	1.79	0.84				
1:35	2.31	1.46	0.81				

into plasma may fall behind, with a rise in the cell/plasma D ratio. Such a set of observations is recorded in the left half of table 3. Usually the arterial cell/plasma D ratio does not rise as high, even with a rapidly falling level, as in this experiment. Sometimes it does not rise at all and usually

does not exceed 0.80. This is in contrast with the findings on the human, where the rate of passage from cells into plasma is so low that $[D]_c^w/[D]_p^w$ may rise from an equilibrium value of 0.3 or 0.4 to 2.0, even though the rate of fall of plasma D from a given level, after cessation of sustaining infusion, is in general slower in man than in the dog. It is thus seen that both *in vitro* and *in vivo* the rate of passage of D from cells into plasma and vice versa is higher in the dog than in man, the rate in each species being much higher *in vivo* than *in vitro*. Details of the findings in man will be published elsewhere.

Contribution of D to urine by cells. Even though the cells contain a large proportion of D, one could still use D plasma clearance as a measure of RPF (if plasma D extraction were complete) provided no significant amount of D moved from cells into plasma during a renal passage. If, however, cell D moves into plasma on D depletion of the latter and becomes available for excretion, then urinary D per minute will be greater than original plasma contribution of D per minute and to this extent D plasma clearance will exceed RPF. That such a cell contribution of D does take place is indicated by the finding that renal vein cell D is always considerably lower than arterial cell D, where plasma D level is below that at which D clearance begins to be self-depressed (about 13 mgm. I per 100 cc.) and where diffusion equilibrium between arterial cells and plasma has been established. When the plasma D level is so high that extraction falls the difference between arterial and renal cell D naturally falls. The average ratio

$$\frac{\text{mgm. D per 100 cc. renal vein cells}}{\text{mgm. D per 100 cc. arterial cells}}$$

at equilibrium plasma levels below 13 mgm. is 0.77 (average of 11 observations), with extremes 0.64 to 0.90. However, renal vein cell D does not fall proportionally to renal vein plasma D; whereas, after equilibration at plasma D levels below that of self-depression of clearance, arterial plasma D is always higher than arterial cell D, renal vein plasma D is always lower than renal vein cell D. This merely means that while 30 to 16 minutes suffice for equilibration, the time of a renal passage does not suffice. An experiment which illustrates most of these points is shown in table 3.

We believe that the observed passage of D from cells into plasma has in large part taken place by the time the blood enters the renal vein rather than during the short time (average 2 to 3 min.) between the beginning of the drawing of the sample and the beginning of centrifugation. The evidence for this is the extremely low rate of passage in the *in vitro* experiments. The statement (2), that the finding that D plasma clearance/D plasma extraction is higher than independently measured RPF is "even more direct evidence of cell contribution of D than is the finding that

renal vein cell D is lower than arterial cell D," is not justified. The *in vitro* experiments, however, do indicate that there is a significant cell contribution of D to the urine.

Completeness of extraction of D from renal vein, blood or plasma. We find that renal extraction, $\dfrac{A-RV}{A}$, of D is never complete from either plasma or cells. When the arterial plasma level is below 13 mgm. I per 100 cc. the plasma extraction has averaged 0.74 (0.61 to 0.85), cell extraction 0.23 (0.10 to 0.36) and whole blood extraction 0.57 (0.46 to 0.66). Within this range of plasma levels (1.3 to 13 mgm. I per 100 cc.) there is but little correlation between plasma D level and completeness of extraction, while
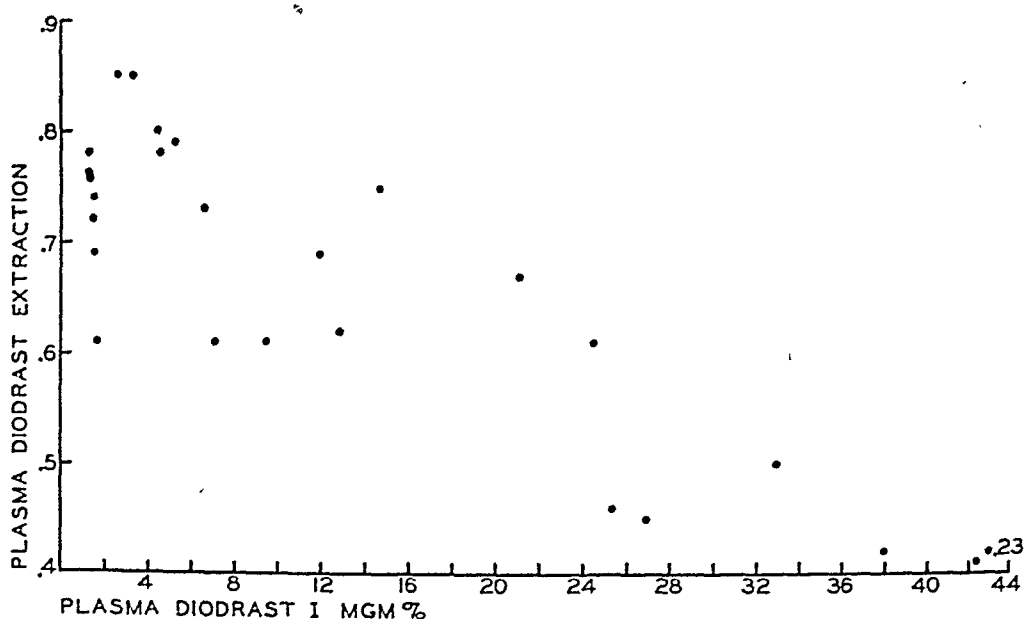
Fig. 1. Effect of varying plasma D level on plasma D extraction. Iodine (I) is a measure of diodrast (D). The anomalous extractions discussed in the text are not included in this figure. The point marked (0.23) designates an extraction of 0.23.

the extraction progressively falls at higher plasma levels. Some dogs consistently show a higher extraction than do others at a given plasma level. Figure 1 shows the relation between arterial plasma D level and plasma extraction; the data are from 4 dogs.

Anomalously low extractions. On one occasion out of a total of 44 renal vein bloods drawn and analyzed for D we have found renal vein plasma D higher than arterial plasma D; this undoubtedly means contamination by urine although none was seen entering the syringe; here the renal vein plasma inulin was also higher than the arterial. On two occasions we have found renal vein plasma D essentially the same as arterial. One explanation for this is that the supposed renal vein blood came from the renal artery. This is believed to be the correct explanation for one of

these cases, since the blood looked arterial. In the other case (K9, 3/28/40, R3), however, it seemed very probable that venous blood was obtained. If this is true, 3 possible explanations suggest themselves; first, that the puncture brings about a temporary suppression, presumably through nervous influences direct or reflex, of D excretion; second, that the needle point was close enough to the vena cava that caval blood to the exclusion of renal vein blood was obtained; third, that just the right amount of urine contamination occurred to bring the renal vein plasma D concentration up to the arterial. The second explanation seems inherently improbable. The third also seems inherently improbable; further light on this possibility may be afforded by the following consideration. If there had been a normal extraction with subsequent contamination by urine there would probably not have been time for the D from urine to come into diffusion equilibrium with the cells, since this would be an *in vitro* process. One would thus expect to find renal vein cell D lower than arterial cell D, as is always the case when a normal plasma extraction is observed. In the case under consideration, however, both plasma and cells of renal vein showed the same D content as arterial plasma and cells, which speaks against urine contamination while being compatible with all of the other possibilities.

In 5 other cases the extraction has been measurable but greatly below the lower limit of what we consider normal at the existing arterial plasma I level. In all 5 of these cases there had been difficulty in getting the renal vein sample. At arterial plasma I levels of 5.1, 7.9, 4.7, 7.9 and 12.6 mgm. the plasma extractions were 0.41, 0.32, 0.15, 0.33 and 0.22, respectively. In all of these cases the observed cell extraction also was very low, the ratio of cell extraction to plasma extraction being essentially the same as when plasma extraction is normal. As in the preceding paragraph, this speaks against contamination by urine as being responsible; we believe they represent partial temporary suppression of D excretion. It seems improbable that they could represent varying admixtures of renal vein and arterial blood, although this possibility is not excluded. The 8 anomalous D extractions discussed in this and the preceding paragraph are not included in figure 1 or in the preceding averages.

Whether such anomalous D extractions actually represent transitory suppression of D, whether this suppression is purely tubular and if so whether it is due to the action of inhibitory fibers to the tubular mechanism or merely to a transitory circulatory deficiency of the tubules cannot be answered without much further work. It is of interest in this connection to examine the simultaneous inulin extractions. If tubular excretion of D were completely suppressed while glomerular filtration was not completely suppressed the plasma extraction of D would be slightly less than that of inulin, slightly less because part of the plasma D is not filterable (11). If the suppression of tubular excretion resulted from activity of inhibitory

fibers to the tubular mechanism, with no circulatory change, inulin extraction should remain normal and should be slightly higher than D extraction. If, on the other hand, an adequate renal afferent vasoconstriction occurred, inulin extraction would fall. In the case (K9, 3/28/40, R3) where renal vein and arterial plasma D were essentially the same (extraction 0.04) the inulin extraction was correspondingly low (0.05). We interpret this as meaning that tubular excretion of D was completely suppressed and glomerular filtration greatly diminished, which suggests that in this case the effects were largely or wholly circulatory. This effect is very transitory, since both D and inulin plasma clearances for that 17 minute period were essentially the same as for another urine period in the same experiment in which D and inulin clearances and extractions were normal. In the 5 cases where D extraction was very low but higher than in the case just discussed, the inulin extraction was considerably lower than the D, indicating that tubular excretion of D had not been completely suppressed. Inulin extraction was itself considerably below normal, indicating partial suppression of glomerular filtration.

The question of transitory suppression of D excretion must be investigated by making urine collections at very short intervals while procedures designed to bring on such suppression are being carried out; in this way transitory falls in D output can be detected. We are not yet ready to report on this point. The question may be raised whether the renal vein puncture method of determining renal blood or plasma flows by observed extractions is satisfactorily reliable. Even though infrequent, the occasional finding of a very low extraction makes one wonder whether the extractions of the order of 0.6 or 0.7 may not represent milder degrees of the suppression process, if such exists. We cannot give a positive answer at this time. In a discussion at the 1940 meeting of the American Physiological Society Dr. Homer W. Smith stated that he and his collaborators had so far invariably observed anomalously low plasma D extractions on human subjects under various anesthetics, extractions so low that they obviously could not represent the mean extractions for the corresponding urine periods. Whatever the explanation may be, the difficulty seems much less likely to appear in the dog than in man.

Our present feeling is that in the dog by a judicious selection one can get D and inulin extractions which will yield a fair approximation to the mean extractions. Thus, if 5 out of 6 samples in one experiment show D extractions between 0.7 and 0.8 while the sixth, which may come between two normals, shows an extraction of 0.3, we feel justified in rejecting the low value and in concluding that the true mean extraction is between 0.7 and 0.8. Furthermore, the fact that D plasma clearance is independent of plasma D level up to a level of about 13 mgm. I per 100 cc., the same as that at which observed extraction begins to fall, indicates that our observed extractions, with the exceptions noted and discussed, are probably

representative of the mean extractions for the corresponding periods, i.e., that true mean extractions behave as do our observed extractions in being independent of plasma level up to about 13 mgm. I per 100 cc.

SUMMARY

The findings of Van Slyke and collaborators a, that urea comes into diffusion equilibrium between plasma and cells very rapidly *in vitro* and *in vivo*; b, that the *in vitro* equilibration of creatinine is very slow and the *in vivo* equilibration is slow enough that renal vein cell creatinine equals arterial cell creatinine, and c, that inulin is absent from blood cells *in vivo*, are confirmed.

The diffusion equilibration rates of D *in vitro* and *in vivo* are studied. The *in vitro* rate of passage of D between cells and plasma is very low. The *in vivo* rate of passage is high enough in the dog that renal vein cell D is lower than arterial cell D. It is concluded that there is a contribution of D to the urine by the cells in the dog.

At diffusion equilibrium the concentration of D per 100 cc. of cell water averages only 58 per cent of that in plasma water, this value being independent of the D level.

At arterial plasma D levels below that at which clearance begins to be self-depressed (about 13 mgm. I per 100 cc.) the extraction has averaged 0.74 (0.61 to 0.85) with but little correlation between completeness of extraction and plasma level between the levels 1.3 to 13 mgm. I per 100 cc. At higher plasma levels the extraction progressively falls. An anomalously low extraction is occasionally observed; possible explanations are considered.

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OBSERVATIONS ON INULIN AND DIODRAST CLEARANCES AND ON RENAL PLASMA FLOW IN NORMAL AND HYPOPHYSECTOMIZED DOGS[1]

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If plasma diodrast (D) extraction, $\dfrac{A\text{-}RV}{A}$, were complete and if no D moved from cells into plasma during a renal passage, D plasma clearance would be a measure of renal plasma flow, RPF. In practice, however, the two factors, incompleteness of D extraction and cell contribution of D (1) (2), work in opposing directions to disturb the relation between D plasma clearance and RPF, the first working to make the clearance lower, the second to make it higher than RPF. If the first factor alone were operative, D plasma clearance/D plasma extraction would be a measure of RPF; in so far as the second factor also is operative, this expression will be higher than RPF. If the cell contribution of D raises D plasma clearance less than the factor of incompleteness of D extraction lowers it, D plasma clearance will be less than RPF; the two factors might be balanced so that D plasma clearance would equal RPF. A comparison of D plasma clearances with simultaneous and independent RPF determinations in the dog is made in this paper. Observations on the relation between plasma D level and clearances and on the tubular excretion of D are also reported.

The general plan of the experiments has been described (2). The dogs were on a diet of Purina dog chow with a weekly supplement (given on Saturday) of 1 pound of raw meat. A previously described method for D (3) was used. Filtrate digestions were hand heated, urine digestions in a water bath. In its present published form the method yields slightly more consistent values on filtrates with hand heating than with the water bath; with urine either method of heating gives complete recoveries. We prefer the filtrates to unprecipitated plasma digestions. A determination of the percentage of D coming through the filtrates is made on each animal at 2 or 3 known levels, i.e., known amounts of D are added to blank blood,

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plasma and cells.   These percentages continue to be remarkably constant.
Inulin was determined essentially according to Corcoran and Page (4).

Relation of plasma D level to D plasma clearance.   Since the extraction
of D has been found independent of plasma D level between the limits 1.3
and 13 mgm. iodine (I) per 100 cc. (2), it follows that clearance should
also be unaffected by changes in plasma level between these limits and this
is found to be the case.   Data on the relation between plasma clearance
and plasma D level are recorded in figure 1.   The higher the plasma level
above 13 mgm., the less the clearance.   The average at levels below 13
mgm. is 246 cc./minute/M² (26 observations on 6 dogs).
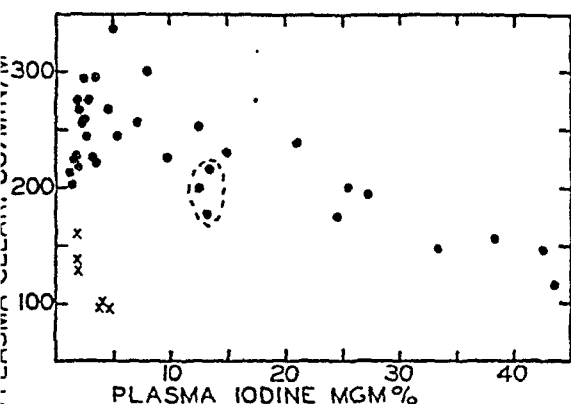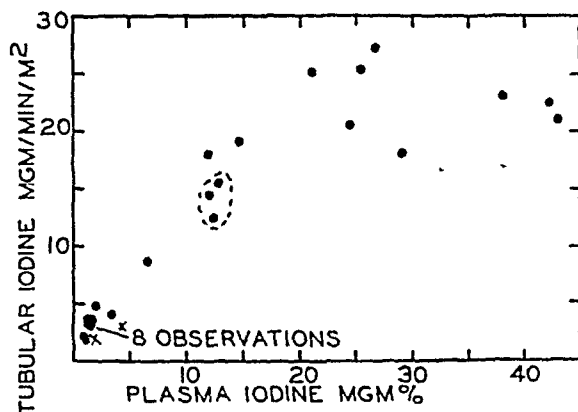
Fig. 1   Fig. 2

Fig. 1. Diodrast plasma clearance as a function of plasma level.   The clearance is
essentially independent of plasma level up to about 13 mgm. I per 100 cc. and falls
progressively with increasing levels above 13 mgm.   The 3 points enclosed by a
broken ring were obtained on a dog, K9, which had unusually small kidneys.   The
values designated by X are on 2 hypophysectomized dogs.

Fig. 2. Tubular output of D as a function of plasma level.   The output is linearly
proportional to plasma level up to about 13 mgm. I per 100 cc. and becomes inde-
pendent of plasma level at about 20 mgm., i.e., diodrast Tm is attained, which has
averaged 22.4 mgm. I/min./M².   The enclosed points are from a dog, K9, which had
unusually small kidneys.   The values designated by X are on 2 hypophysectomized
dogs; each represents the average of 3 observations.

Relation of plasma D level to tubular excretion of D.   By means of simul-
taneous inulin clearances and calculations of the filterable fraction of D
(5) one can determine the glomerular and thus the tubular excretion of D.
The rate of tubular excretion, in mgm./min./M², rises linearly with rising
plasma D level up to a level of about 13 mgm. I per 100 cc.; this is in agree-
ment with the extraction and clearance findings.   Above this level the slope
falls and the curve becomes horizontal at 18 to 20 mgm. I per 100 cc., i.e.,
the maximal rate of tubular excretion of D (Tm) is attained.   Data are
reported in figure 2.   Diodrast Tm averages 22.4 mgm./min./M², which
is not much different from our figure of 25 mgm./min./M² on man (6).

Comparison of D plasma clearances with simultaneously determined RPF. Where V_p is the plasma fraction of the hematocrit reading (all bloods heparinized), RPF was determined by two methods simultaneously,

(a) $$\frac{\text{Inulin plasma clearance}}{\text{Inulin plasma extraction}},$$

(b) $$\frac{\text{D whole blood clearance} \times V_p}{\text{D whole blood extraction}}.$$

Whole blood D was determined from plasma and cell D analyses and hematocrit readings; this was occasionally checked by direct whole blood
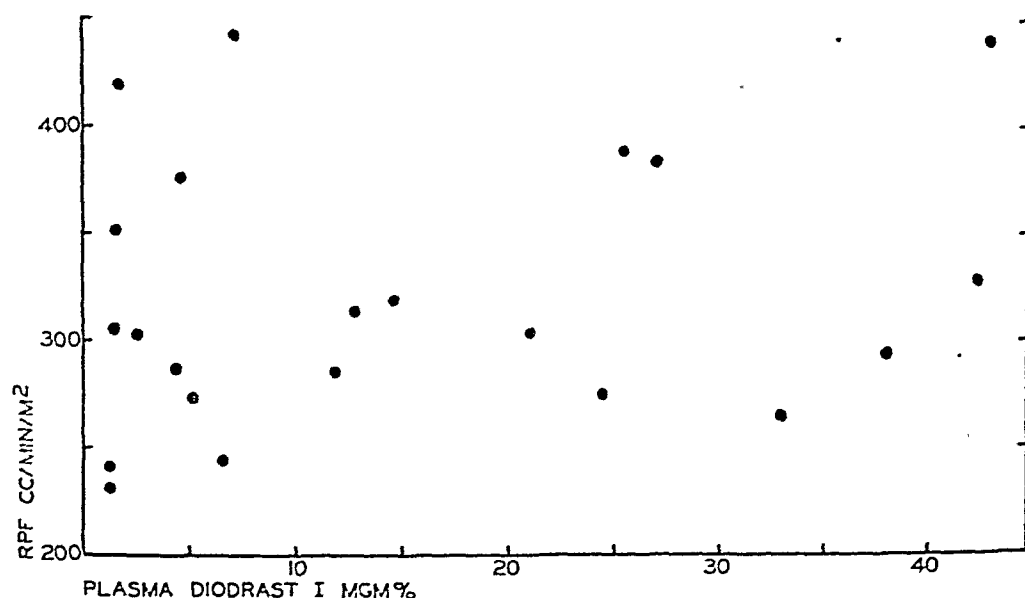
Fig. 3. True RPF values on 6 normal dogs, averaging 325 cc./min./M². As might be expected, these values are independent of plasma D level.

analyses. With observations on 22 periods, the average RPF values in cc./min./M² are, (a) 323 (215 to 436), (b) 326 (202 to 442). Data on RPF are shown in figure 3; the values are independent of the plasma D level. The recorded values are averages of the inulin and D values, which usually differ by less than 10 per cent. The D plasma clearance averaged 246 cc./min./M² when the plasma I level was below 13 mgm. per 100 cc., and the average D plasma clearance/D plasma extraction was 363 cc. (263 to 510). The latter average is higher than RPF (325 cc.) because of the cell contribution of D to the urine. Since 363 is 112 per cent of 325, it follows that for every 100 mgm. D contributed to the urine by the plasma, 12 mgm., on the average, are contributed by the cells. This is not a large but is a significant contribution. Evidence has already been advanced

(2) that this is a real contribution to the urine and not due merely to the passage of D from cells into plasma after the kidney has been passed.

The D plasma clearance is lower than RPF because the factor of incompleteness of extraction overbalances that of cell contribution. The average of all RPF values (22 periods), 325 cc., is 1.32 times the average of all D plasma clearances at plasma I levels below 13 mgm. (26 periods), 246 cc. However, of the 22 RPF periods only 13 were carried out at plasma I levels less than 13 mgm.[2] The average of these 13 RPF periods is 313 cc. and the average of the 13 simultaneous clearance values is 263 cc. Thus, in this series of 13 simultaneous determinations at plasma I levels less than 13 mgm., the average RPF is 1.19 times as great as clearance. This multiplier, however, has varied between 1.03 and 1.58, with no correlation between value of multiplier and plasma I level between the limits of 1.3 and 12.9 mgm. Variability of extraction is the factor chiefly responsible for variations in the multiplier.

The average renal whole blood flow is 655 cc./min./M^2, or 22.3 per cent of Marshall's (7) value of 2940 cc./min./M^2 for cardiac output in the dog. Corcoran and Page (8) found an average RPF of 250 cc./min./M^2 and RBF of 460 cc./min./M^2, using inulin and phenol red extractions. They used dogs from which the opposite kidney had been removed, with about 3 months allowed for hypertrophy of the explanted kidney. All of our dogs had both kidneys; the ratio of our values to those of Corcoran and Page is about what would be expected.

Filtration fraction. Inulin plasma clearance has averaged 91 cc./min./M^2. This is 28 per cent of average RPF; this filtration fraction has varied between the limits 0.18 and 0.40. The filtration fraction as indicated by the inulin extraction agrees well with the value obtained by inulin clearance/RPF, where RPF is D blood clearance $\times V_p$/D blood extraction, i.e., the inulin and D findings are in agreement. In this series no experimental procedures designed to affect the filtration fraction have been carried out. The extreme value of 0.40 may be somewhat suspect, although it is difficult to see what technical difficulties could give erroneously high extractions; any accident to the renal vein blood should lower extraction. Our average of 0.28 is close to Corcoran and Page's average of 0.297 and slightly higher than Van Slyke, Hiller and Miller's (9) average of 0.223.

Table 1 presents data on a few periods; most of the points discussed are illustrated in this table.

Hypophysectomized dogs. In figures 1 and 2 are shown a few observations on D plasma clearance and tubular excretion in 2 hypophysectomized

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[2] The remaining 13 of the 26 clearances at plasma I levels less than 13 mgm. were not accompanied by RPF determinations.

dogs. It is seen that both tubular output and plasma clearance are about half as high as in the normals. Inulin clearances (not shown in figures)

TABLE 1

Data of 5 periods on clearances and renal flows

A and RV refer to arterial and renal vein specimens, respectively. Uncorrected cell I means milligrams diodrast iodine per 100 cc. of centrifuged cells. Whole blood I is calculated from plasma and cell analyses and hematocrit readings. Mean D extraction is obtained from mean D levels for the respective urine periods, as determined by plotting analytical values against time. Glomerular D plasma clearance is obtained by multiplying inulin clearance by the filterable fraction of D as obtained from the constants of Smith and Smith (5); tubular clearance is total clearance minus glomerular clearance. Column (23) is to be compared with the average of columns (17) and (22), the excess representing cell contribution of D. Tubular I (24) is total I output minus glomerular I output as determined from inulin clearance and filterable fraction of D.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
DOG	URINE FLOW	PLASMA I		UNCORRECTED CELL I		WHOLE BLOOD I		MEAN D EXTRACTION		
		A	RV	A	RV	A	RV	Plasma	Cells	Blood
	cc./min./M²	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent			
K1	5.16	1.34	0.32	0.64	0.46	0.94	0.40	0.76	0.24	0.57
	5.31	1.32	0.32	0.64	0.46	0.93	0.40	0.76	0.24	0.57
K3	4.51	1.70	0.67	1.0	0.79	1.35	0.73	0.61	0.29	0.46
	1.56	1.55	0.49	0.91	0.56	1.25	0.52	0.69	0.36	0.58
	1.20	1.52	0.39	0.87	0.54	1.24	0.45	0.74	0.36	0.64

(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)
D PLASMA CLEARANCE			D BLOOD CLEARANCE	RBF $\frac{(15)}{(11)}$	RPF AS $\frac{(16)}{V_P} \times$	PLASMA INULIN		MEAN PLASMA INULIN EXTRACTION	INULIN PLASMA CLEARANCE	RPF as $\frac{(21)}{(20)}$	$\frac{(12)}{(9)}$	TUBULAR I
Total	Glomerular	Tubular				A	RV					
cc./min./M²	cc./min./M²	cc./min./M²	cc./min./M²	cc./min./M²	cc./min./M²	mgm. per cent	mgm. per cent		cc./min./M²	cc./min./M²	cc./min./M²	mgm./min./M²
213	61.2	151.8	304	534	229	64.3	44.5	0.308	77.8	253	280	2.04
202	62.8	139.2	286	502	215	64.0	43.3	0.324	79.6	246	266	1.84
293	79	214	370	800	406	42.4	32.5	0.234	101	432	480	3.63
270	73	197	332	673	370	46	32.6	0.292	96.8	332	390	2.97
276	81	195	338	530	297	49.1	33.0	0.328	103	313	370	2.96

are also about half the normal. It thus follows that both glomerular and tubular outputs of D are about half the normal. We do not yet have observations on tubular outputs at high plasma D levels, Tm, on these

animals but this will probably also be low. The primary purpose in undertaking our inulin and D observations was to get further information on our earlier finding (10) that for several weeks after hypophysectomy or after the production of diabetes insipidus by appropriate hypothalamic lesions, the creatinine clearance was greatly reduced. The question arose as to whether this meant a low RPF or a low filtration fraction or both. We do not yet have observations on extractions or RPF (no renal explant hypophysectomized dogs), but the present finding that the ratio of inulin clearance to D plasma clearance is about normal indicates that the filtration fraction is normal and the low D plasma clearances probably mean a low RPF. Further observations on tubular function and on true RPF in these types of animals are in progress.

DISCUSSION. It is seen that in the dog with one kidney explanted and the other present D plasma clearance is definitely less than RPF at plasma I levels between 1.3 and 13 mgm. per 100 cc. In the series where direct comparison was possible RPF averaged 1.19 times clearance. In general it may be said that if one multiplies the D plasma clearance (at plasma levels between the above limits) by 1.2 he will get a value which usually will differ from RPF by less than 10 per cent.

SUMMARY

Diodrast plasma clearance in dogs has averaged 246 cc./min./M² at plasma I levels between 1.3 and 13 mgm. per 100 cc.; the clearance falls progressively at higher levels.

Tubular output of D rises linearly with increasing plasma level up to about 13 mgm. I per 100 cc.; here the slope begins to fall and the curve becomes horizontal at about 20 mgm. Diodrast Tm, being attained at a plasma level of 20 mgm. I, averages 22.4 mgm. I/min./M².

True RPF is obtained, usually within 10 per cent, by multiplying D plasma clearance, obtained at plasma I levels between 1.3 and 13 mgm., by 1.2. Renal plasma flow has averaged 325, renal blood flow 655 cc./min./M².

About 11 per cent of the D entering the urine comes from the blood cells.

The filtration fraction of plasma has averaged 0.28.

The inulin and D plasma clearances and the rate of tubular output of D in the hypophysectomized dog are about half the normal. This indicates a low RPF with an essentially normal filtration fraction.

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FREQUENT FAILURE OF A SINGLE INSEMINATION TO ACTIVATE THE CORPORA LUTEA OF THE RAT SUFFI-CIENTLY FOR IMPLANTATION OF FERTILIZED OVA[1]

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In a previous communication (Ball, 1934) it was shown that pseudo-pregnancy in the intact rat followed sterile mating invariably only if two or more vaginal plugs had been deposited. In about one-third of the cases in which mating was terminated after the formation of the first plug estrous smears recurred within 4 or 5 days as if no mating had taken place. In the light of this fact it seemed probable that a similar failure of the pseudo-pregnancy mechanism might prevent the implantation of healthy, fertilized eggs after mating with fertile males if only one plug were formed. A series of one-plug matings was therefore undertaken in order to discover if such a failure does in fact occur when the ova are fertilized as well as when they are not.

EXPERIMENTAL PROCEDURE. Seventy-three female rats of a mixed hooded strain were used for these matings. They were between 3 and 4 months old at the beginning of the experiment. Most of them had borne one litter but the litters had been discarded and the mothers were started on the experimental breeding after a rest of not less than 10 days. They were fed Purina Dog Chow and lettuce with additional dried whole milk during pregnancy. They were, of course, kept segregated except during the observed matings.

When vaginal smears indicated probable sexual receptivity each female was allowed to copulate with a male of proved fertility until one plug had been formed. She was then returned to her home cage. Vaginal smears were taken daily after this until the appearance of either the red blood cells indicative of pregnancy or cornified cells indicating a new estrus and failure of gestation (Evans and Burr). In the former case smears were discontinued and the animal was set aside as pregnant. If, however, an estrous smear appeared first she was mated again, usually to the same male, and this time three plugs were permitted before the animals were sepa-

rated. In about half of these three-plug matings a vasectomized male was substituted for the fertile male after the first plug had been deposited in order to show that the improved reproductive performance after three-plug matings over that resulting from one-plug matings was not due to the presence of a greater concentration of spermatozoa in the female sex tract. Since the results were the same whether fertile or sterilized males were used for the second and third plugs all cases have been tabulated together for the following discussion.

After the three-plug mating the female was treated as she had been after the one-plug mating. If she again failed to become pregnant she was considered sterile and her one-plug record was discarded although occasionally such a female would produce a normal litter if mated again.

The litters born after these matings were discarded at birth and the same procedure was repeated once more after a rest period of 10 days or more.

Many of the single plugs were examined microscopically and in every case active spermatozoa were found in normal profusion.

A second group consisting of 19 rats of an albino strain were treated in exactly the same way. These animals are reported as a second group only because their performance was somewhat different. They were the same age as the hooded rats, were bred during the same months and were kept in the same cages. They were not considered a separate group until it was discovered that all but one had become pregnant after one-plug matings, a much higher fertility than that of the hooded animals.

RESULTS. In the first group, the hooded animals, there were 109 one-plug matings in which the fertility of both male and female was proved by production of a litter as a result of either that mating or the subsequent three-plug mating. Of these 109 one-plug matings, 71 (65 per cent) resulted in litters while 34 (31 per cent) were followed by estrus in 4 or 5 days as if no mating had occurred, showing that in about one-third of the cases one plug is not enough to stimulate the corpora lutea sufficiently to permit implantation. In only 4 cases (4 per cent) did these one-plug matings fail to produce litters for reasons other than failure of the mechanism which produces pseudopregnancy in sterile mating. In each of these 4 cases (1 resorption and 3 pseudopregnancies) the reproductive history of the animal offered some other evidence, such as very small litters or too long non-copulatory cycles, that her reproductive system was not quite up to normal.

In no case did a 4-day or 5-day cycle follow a three-plug mating. If pregnancy failed, the animal was always at least pseudopregnant. In other words, three plugs were always an adequate stimulus to produce functional corpora lutea, although one plug was often not. The outcome of these matings was, therefore, almost precisely what had been expected in the light of the previous experience in mating hooded rats with vasec-

tomized males, even the two to one proportion of success and failure remaining the same for the hooded animals.

The albinos, however, were apparently more reactive to the stimulus of copulation for in this group 31 (94 per cent) of the 34 one-plug matings resulted in litters and only 2 (6 per cent) of them (both in the same female) were followed by short cycles. There was one pseudopregnancy.

The albino rats had been raised in another laboratory and 27 of the hooded animals had come from a third laboratory so that it cannot be said with certainty that the difference in performance was due to strain difference but this seems the most probable explanation since all animals appeared to be in good health at the time of the experiment. A difference between these hooded rats and another albino strain in responsiveness to injected hormones has already been noted (Ball, 1939).

Although there would seem to be no reason to doubt that the eggs were fertilized by a single plug as well as by three since one fertile plug was as successful as three in producing young if it was followed by infertile plugs, nevertheless several females were examined when they showed estrous smears four days after mating. To obtain the eggs one uterus was carefully tied off as close to the cervix as possible and then removed with its contents and with the ovary attached. The other uterus and ovary were left intact in order to test fertility as usual by three-plug insemination. The contents of the removed uterus and tube were examined by Dr. Carl G. Hartman to determine the presence and condition of eggs.

A number of the animals subjected to this examination proved to be sterile when tested later by three-plug matings. These cases, like the other cases of sterile animals, were of course discarded since it was the purpose of this experiment to determine the relative number of successful pregnancies after one-plug and three-plug matings. In order to do this it was obviously necessary to use only animals that were capable of producing litters, given adequate stimulus. It is, however, of interest to note that the eggs found in the sterile animals were uniformly unfertilized and disintegrating.

In contrast to this defectiveness of the gametes in the animals discarded as sterile, the eggs found in the four fertile females examined were fertilized and developing normally showing that it was not the zygotes but the maternal endocrine system which was responsible for the failure of pregnancy in these cases.

. It is apparent from these studies that for some rats a single insemination is insufficient stimulus to insure inception of the chain of events necessary to prepare the uterus for implantation and that this process is quite independent of the fertilization and early growth of eggs. Failure of pregnancy may be due to either or both. If only gametes fail, pseudopregnancy results. If only preparation of the uterus fails, due to too little cervical

stimulation, the next estrus is not inhibited and normally developing eggs are contained in a proestrous uterus.   If both fail there is a short cycle but the proestrous uterus contains only disintegrating eggs.   Deposition of three plugs prevents the occurrence of the second and third of these conditions.   It may be added that rats usually make three or more plugs if they are simply left together over night when the female is in heat.   But occasionally only one plug will be made due to low sexual excitability of one or both partners.

SUMMARY

Approximately one-third of 109 single-insemination matings of fertile rats were followed by estrous smears within 4 or 5 days as if no mating had taken place.   Normally developing fertilized eggs were found in the uterus of four such animals examined at the time of proestrous smears four days after mating.

This frequent failure of pregnancy after deposition of a single vaginal plug is considered to be due to the same failure of the pseudopregnancy mechanism that had occurred in a comparable proportion of cases when similar matings had been made with vasectomized males.   Since short cycles never occurred after the deposition of three plugs by either fertile or sterile males it is concluded that for the animals used in these experiments, a mixed hooded strain, one plug is insufficient stimulus to insure inception of the chain of events necessary to prepare the uterus for implantation whether there are eggs to implant or not.

A small group of rats of an albino strain responded to the stimulus of one plug much more readily than the hooded animals.

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THE REACTIONS OF PARTIALLY DENERVATED SMOOTH MUSCLE TO ADRENALINE AND SYMPATHIN

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Although it is generally agreed that skeletal muscle is composed of motor units which may contract independently of one another, it is still doubtful whether there are similar units in smooth muscle. Since the bulk of the histological evidence favors a sparseness of innervation resulting in one nerve fiber to more than one smooth muscle cell (for references see Cannon and Rosenblueth, 1937), the existence of units composed of single smooth muscle cells is improbable.

Denervation of the nictitating membrane results in an increased sensitivity to adrenaline and to sympathin (see Cannon and Rosenblueth, 1937). This hypersensitivity decreases and disappears as regeneration of the previously severed postganglionic fibers progresses (Simeone, 1937).

Lawrentjew and Borowskaja (1936) have shown histologically that section of some of the postganglionic fibers distributed to a smooth muscle effector results in a partial denervation. It seemed plausible that partial denervation of the nictitating membrane might sensitize only some cell groups. Such a result would support the concept of a relative unitary organization of smooth muscle. The present study was undertaken to answer this question.

METHOD. Cats were used. One superior cervical ganglion was exposed aseptically under ether anesthesia and some of the postganglionic fibers were severed. For control observations the ganglion of the opposite side was either removed, preganglionically denervated, or left intact. Seven to 14 days were allowed for sensitization to develop. The cats were then used under dial anesthesia (Ciba, 0.8 cc. per kgm. intraperitoneally).

Isotonic contractions were recorded from one or both nictitating membranes by levers providing a 20-fold magnification and a load of 3.5 grams. Injections of adrenaline (adrenalin, Parke, Davis and Co.) were made into the femoral vein in dilutions of 1:50,000 to 1:200,000 in 0.9 per cent NaCl. One dilution was employed in any series of observations. A constant time (10 sec.) was used for each injection. For nerve stimulation shielded electrodes were placed on either the pre- or postganglionic fibers of the

cervical sympathetic. Maximal shocks were delivered at various frequencies from a multivibrator circuit discharging through a transformer. Each frequency was applied for a constant time (20 sec.).

RESULTS.   A. *Responses to adrenaline.*   Series of responses of partially denervated nictitating membranes to varied doses of adrenaline were recorded simultaneously with the responses of control membranes, normal or with total pre- or postganglionic denervation. Adrenaline was given in either gradually increasing or gradually decreasing amounts. Random doses did not yield consistent results. This inconsistency is probably due to changes in the sensitivity of the muscle cells during the course of the
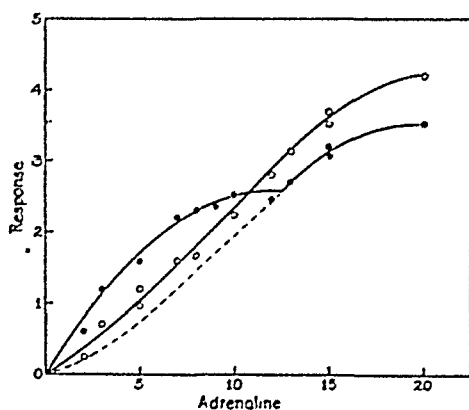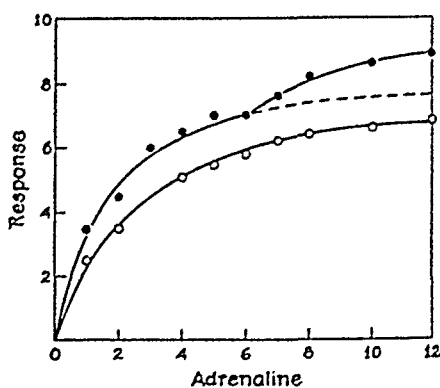
Fig. 1   Fig. 2

Fig. 1. Dose-response curves for adrenaline. Ordinates: maximal height of responses. Abscissae: adrenaline (γ). Dots: partially denervated nictitating membrane; the broken line is the inferred extrapolation of the upper segment of the curve. Circles: normal control membrane of the opposite side.

Fig. 2. Dose-response curves for adrenaline. Ordinates and abscissae as in figure 1. Dots: partially denervated membrane; the broken line is the inferred extrapolation of the lower segment of the curve. Circles: totally denervated control membrane of the opposite side.

experiment, for injections of adrenaline result in a gradual desensitization of innervated muscles (Simeone, 1938).

The control membranes yielded smooth, continuous curves when the maximal height of response was plotted against the corresponding dose of adrenaline (Rosenblueth, 1932; figs. 1 and 2). The curves from the partially denervated muscles, on the other hand, invariably showed a discontinuity or break (figs. 1 and 2). The level in the curve at which this break occurred varied from one animal to another, probably because the fraction of postganglionic fibers interrupted by the previous section was also variable (see below).

An examination of figures 1 and 2 reveals that the partially denervated membrane responds to relatively large doses of adrenaline like the normal

control (upper segment of the broken curve, fig. 1), whereas it reacts to small doses like the totally denervated control (lower segment, fig. 2). The breaks can be readily interpreted if it is assumed that partial denervation leads to sensitization of a fraction of the total number of cells in the muscle. The lower segment of the curves would then be due almost exclusively to a contraction of the sensitized elements. Large doses of adrenaline would, on the other hand, activate not only the sensitive but also the normal elements.

B. *Responses to preganglionic nerve stimulation.* Series of responses were obtained with various frequencies of nerve stimulation at constant intensity. The magnitude of the response was measured at the end of 20 sec. The results of a typical experiment are plotted in figure 3. As in the case of the curves plotted from the response to adrenaline, the present
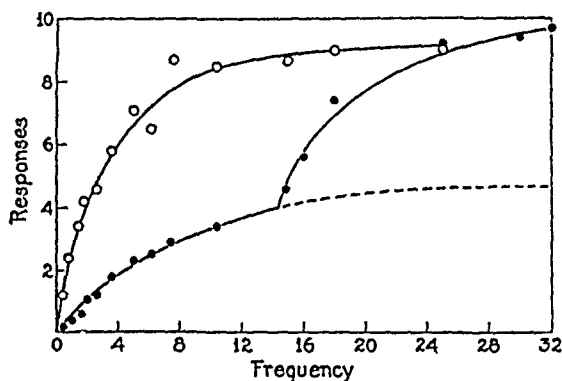
Fig. 3. Frequency-response curves. Ordinates: height of responses after 15 sec. of stimulation. Abscissae: frequency of maximal stimulation of the preganglionic sympathetic fibers. Dots: partially denervated membrane. Circles: normal control membrane.

curves were not smooth but showed a break. The position of this break varied from animal to animal, depending probably on the number of postganglionic fibers cut. In any given animal the break occurred at the same height of response in successive curves built by successive series of observations, but not at the same frequency. This shift of the critical frequency may be explained as due to a progressive exhaustion of the sympathin liberated and to a relative desensitization of the denervated cells (see Simeone, 1938).

The curves in figure 3 differ from the smooth curves obtained by Rosenblueth and Rioch (1933) in acute partially denervated membranes. The difference may be attributed to the increased sensitivity to sympathin which chronic denervation produces in some cells. Thus it is probable that the initial portion of the broken curve in figure 3 represents the response mainly of innervated cell units, while the final portion represents the addi-

tion of denervated (i.e., sensitized) cell units activated by the diffusing mediator. The position of the break in the curve may accordingly be taken as an indicator of the critical concentration of mediator at which a significant diffusion begins.

C. *Postganglionic nerve stimulation.* In 3 cats response-frequency curves were plotted by stimulating first the preganglionic and later the postganglionic fibers. The results were similar for both types of stimulation—i.e., the curves showed a break at approximately the same height of response. It may be inferred that the breaks are to be attributed to some condition in the effector, not in the ganglion.

D. *Effects of removal of the eyeballs.* Brücke (1938) reported that the increased sensitivity to adrenaline of the preganglionically denervated membrane disappears after removal of the eyeball. He inferred that the smooth muscle of the membrane is not sensitized by such denervation, and that the increased response obtained with the eyeballs intact is due exclusively to a greater reactivity than normal of the smooth muscle which protrudes the eye.

Rosenblueth and Cannon (1939) were unable to confirm Brücke's report. In the present study 2 cats were tested as follows. With the eyeballs intact as usual a response-frequency curve for preganglionic nerve stimulation and a response-dose curve for adrenaline were constructed. The eyeballs were then excised and the observations were repeated. The results were uninfluenced by the removal of the eyeballs.

E. *Comparison of the adrenaline and of the nerve stimulation curves.* In the broken curves obtained with adrenaline (fig. 1) the lower segment was attributed to the sensitized and the upper segment to the normal cells. Conversely, in the curves plotted from responses to nerve stimulation, the lower segment was interpreted as due to the contraction of the innervated elements, and the upper segment to the addition of the denervated elements. If these inferences are correct it follows that when only a small fraction of the nerve fibers are cut, the break in the curves should be at a relatively low level of response for adrenaline and at a relatively high point for nerve stimulation. The converse should be true if a large fraction of the nerve fibers are interrupted. These corollaries were supported by the experiments. Whenever the break in the adrenaline curve was low, that in the frequency curve was high and *vice versa*.

DISCUSSION. The results (figs. 1, 2 and 3) indicate that degeneration of some of the postganglionic fibers which supply the nictitating membrane cause a relatively sharp division in the muscle. Some cells retain their normal sensitivity to sympathin and adrenaline while others are more sensitive. Such differentiation is absent in normal or totally decentralized or denervated membranes.

Rosenblueth and Rioch (1933) showed that stimulation of a fraction of

the total nerve supply of a normal membrane tends to activate all the muscle cells if the frequency is sufficiently high. Rosenblueth and Morison (1934) showed that diffusion of sympathin into the blood stream occurred only when the stimulating frequency reached a critical value (3 to 12 per sec.). The present observations agree with these reports. When the frequency of stimulation attains a critical value (4 to 14 per sec.), a diffusion of sympathin takes place from the innervated into the denervated cells. The difference between the present data and those of Rosenblueth and Rioch (1933) lies in the fact that the diffusing sympathin reaches elements whose sensitivity is increased by previous denervation.

If the suppression of a continuous tonic delivery of sympathin is the factor responsible for sensitization after denervation, it may be inferred that the tonic discharge of nerve impulses occurs normally at relatively low frequencies, insufficient for significant diffusion into neighboring elements, since only some cells are sensitized when the denervation is partial.

The bearing of the present data on the problem of the existence or lack of existence of motor units in smooth muscle is complex. Since some elements in the muscle are differentially affected by partial denervation, it is apparent that a relative division into units is defensible. Such a division is obviously in disagreement with Bozler's (1939) recent suggestion that smooth muscles are a single unit of syncytial structure, like the heart. On the other hand, since with sufficiently high frequencies of stimulation the whole muscle may be affected by only some of the nerve fibers it is also obvious that there is no unitary arrangement similar to that of skeletal muscle, as Eccles and Magladery (1939) have suggested.

The most tenable view at present is that the smooth muscle of the nictitating membrane is neither like the heart nor like striated muscle, but occupies an intermediate position. The number of smooth muscle cells controlled by a nerve fiber is variable, increasing when the frequency of the nerve impulses increases (Rosenblueth and Rioch, 1933). With low, physiological frequencies the sphere of action of a nerve fiber would then be relatively small. Section of some nerve fibers does not modify this low frequency. Increased sensitivity becomes apparent in only some muscle cells.

SUMMARY

Chronic partial postganglionic denervation of the nictitating membrane divides the smooth muscle into groups of cells which react in a quantitatively different manner to adrenaline and nerve stimulation.

This difference is due to changes in the effector and not in the superior cervical ganglion.

This action is independent of the presence of the eyeballs.

An explanation of the results is given which entails a clearer definition of the smooth muscle units of the nictitating membrane.

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EXCITABILITY OF THE EXCISED AND CIRCULATED FROG'S SCIATIC NERVE

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The passage of an electrical current through a nerve places it in a state of altered excitability which was called electrotonus by the discoverer of the phenomenon, DuBois Reymond (1849). Pflüger (1859) studied the effects of constant current on nerve with extreme care and found that during the passage of the current there was a lowering of the threshold to induction test stimuli at and near the cathode, while the threshold to such stimuli was raised at and near the anode. The magnitude of the effect was found to be a function of the proximity of the point tested to the constant current electrode and of the strength of the applied current. When the constant current was interrupted the effects at both anode and cathode were reversed. Pflüger concluded that the electrotonic changes of excitability were the essential phenomena of excitation. Some recent workers, Bishop and Erlanger (1926), Bishop (1928a, b), Erlanger and Blair (1932), Bogue and Rosenberg (1934), Rosenberg (1937), associate the excitation phenomena with the development of electrotonus at the cathode. Hill (1936) and his co-workers have, however, postulated the existence of excitatory processes which are independent of electrotonic phenomena.

As experimental data have accumulated, theories of excitation have been developed in an attempt to coördinate the data. Among them may be mentioned the theories of DuBois Reymond (1849), Nernst (1908a, b), Lapicque (1926), Blair (1932), Rashevsky (1933), Monnier (1934) and Hill (1935, 1936). All the earlier ones have proven inadequate since they apply only to particular cases. The theories of Hill, Monnier and Rashevsky attempt to treat mathematically the general phenomena of excitation in terms of two processes. At the cathode one process is rapid, tending to excitation, and the other is slower, giving a recovery, inhibition, adaptation or accommodation, while opposite effects are produced at the anode. Offner (1937) and Young (1937) found that where the intensity-time relations for any type of stimulating current were concerned the theories of Rashevsky and Hill were identical. Rushton (1937)

showed that the same second order differential equation expressed the assumptions of both Hill and Monnier. When extended to include the formulations of Rashevsky and Young (1937), the equation becomes

$$\frac{d^2\theta}{dt^2} + (k_{11} + k_{22})\frac{d\theta}{dt} + (k_{11}k_{22} - k_{12}k_{21})\theta$$

$$= (B - A)\frac{dI}{dt} + [A(k_{21} + k_{22}) - B(k_{11} + k_{12})]I, \quad (1)$$

where θ is the difference between the excitability at any time and that at rest, $\theta = (U - V) - (U_o - V_o)$ as given by Hill, k_{11}, k_{12}, k_{21} and k_{22} as given by Young are velocity constants, and I is the applied current. From Hill, $k_{11} = -1/k$; $k_{22} = -1/\lambda$; $k_{12} = 0$; $k_{21} = 1/\beta$; $A = C$; $B = 0$, and similar identifications are found from Rashevsky and Monnier. It is obvious by inspection that the term in I is necessary for an electrotonus to be established after a sufficient length of time, but this term also requires an excitation by sufficiently large currents—no matter how slowly they rise (cf. Cole, 1933).

All the authors have treated the minimal current gradient by making the coefficient of I zero and excluding electrotonus from the formulation. These theories require that the threshold be independent of any constant direct current and that the alternating current threshold approach infinity as the frequency approaches zero, while the threshold for anode break must be the same as for cathode make. According to the theories a break response must occur after the current has flown for some time, 200 msec. or longer. The absence of a break response cannot be explained by the recent theories.

An extensive group of experiments performed on the circulated frog's sciatic-gastrocnemius showed that it was nearly impossible to secure break responses after constant currents had flowed for intervals of one quarter to five seconds. Break responses were obtained only with currents of injurious strength or after the circulation had been impaired. In the same preparations the make responses were usually tetanic even with stimuli of threshold strength. These responses showed that the constant current was effective as a stimulus over relatively long time intervals. Such results suggested the study of the action potentials of the nerve itself under similar conditions. The cathode ray study showed that the nerve was responding repetitively to the constant current. These divergences from the theoretical predictions and the frequently recorded responses of excised nerve (Bishop and Erlanger, 1926; Bishop, 1928b; Erlanger and Blair, 1932), indicated the desirability of studying the adequately circulated sciatic nerve when subjected to the passage of constant current.

MATERIALS AND METHODS. The test shock method of Bishop (1928b) and Erlanger and Blair (1932) was used to measure the excitability of the alpha fibers of the frog's sciatic nerve during and after the passage of direct current. The Pickerel frog was used exclusively. All frogs were equilibrated to temperatures between 20° and 23°C. for at least one week before experimentation and all data were obtained at the same temperatures. For stimulation and recording the action potential the nerve was either exposed in the animal, or excised and placed in a moist chamber as desired. In the circulated animals, spinal section was performed under ether anesthesia two or more days before use. Hemorrhage was kept minimal.

The constant currents (two megohms in series with the stimulating electrodes) and test shocks were applied through the same pair of electrodes spaced one centimeter apart. A relay that opened a series of contacts tripped the cathode ray sweep and applied both stimuli. A suitable micrometer adjustment permitted the application of the test stimulus at any time from 2 msec. before to 15 msec. after the make of the constant current. Silver-silver chloride electrodes were used for stimulation. An accessory circuit, which included a galvanometer that could be short-circuited, permitted balancing any polarization potential at the stimulating electrodes. After conduction through about 3 cm. of nerve the action potentials were led to a resistance-capacity coupled amplifier, provided with a differential input (Toennies, 1938), which amplified them for observation and measurement on the oscillograph.

The half maximal alpha spike (Erlanger and Gasser, 1924, 1937), served as the index of nerve activity. The test voltage required to evoke the half maximal response was recorded and the rheobasic constant current determined. Then the constant current was adjusted to the desired sub-rheobasic value and applied together with a test shock at the desired time in relation to the constant current make. The test voltage was now adjusted to just produce a half maximal response in the presence of the constant current. The required test voltage was expressed as percentage of the test voltage necessary to evoke a half maximal response when applied alone.

EXPERIMENTAL RESULTS. *Excised nerves.* Nerves that were removed from the animal and immediately placed in a moist chamber showed clearly the inability of the constant current to maintain a constant level of altered excitability with increasing durations of current flow. The adjustment of the nerve to the constant current was, however, very seldom complete; that is, the excitability remained at a level different from the resting condition throughout the time of current passage. Figure 1 shows clearly the magnitude and time course of these excitability changes at both electrodes as functions of the applied constant current strength. It

should be recalled that at the cathode the excitability is increased while at the anode it is decreased. The maximum effect of the subrheobasic constant current is attained between 0.5 and 1 msec. after its make and the effect begins to decline after about 2 msec. of current flow. This decay of the electrotonic state may be considered as an inhibition, adaptation, or accommodation.

In order to establish a method of measuring these variations of the electrotonic state it is necessary to consider briefly the data from circulated nerves (fig. 3). When, in the circulated nerve, the strength of the test
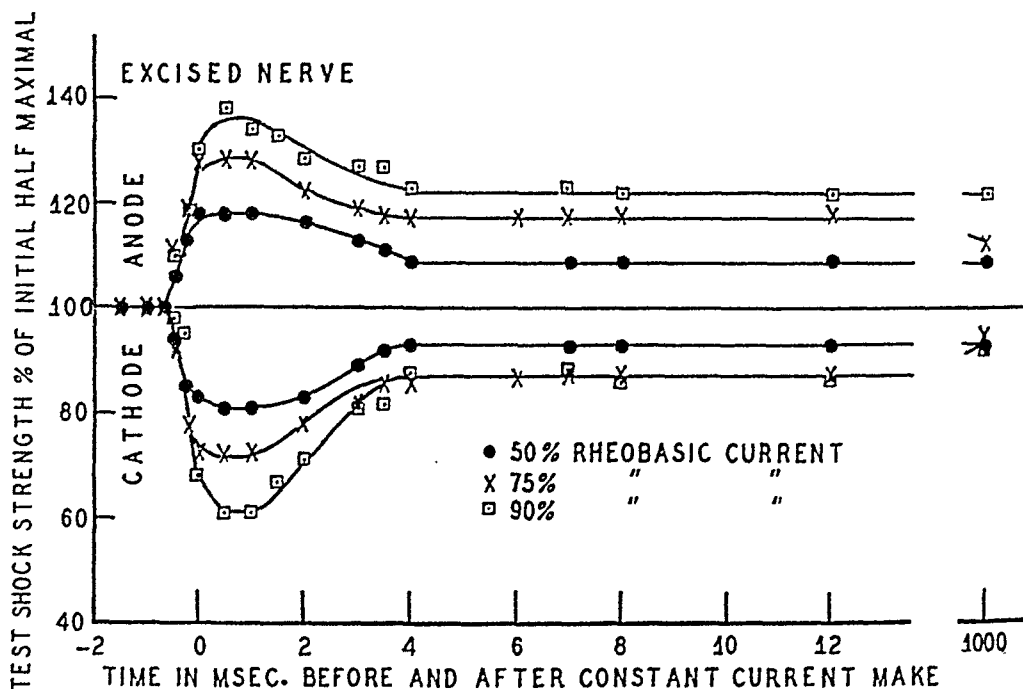
Fig. 1. Excised nerve. Time course of excitability at anode and cathode of subrheobasic constant currents, as measured by the test shock method.

Abscissae: Time of application of test shock in relation to constant current make.

Ordinates: Test shock strength as percentage of the test shock which, when applied alone, evokes a half maximal alpha action potential.

shocks required to evoke a half maximal alpha spike, in the presence of constant currents, is plotted against the strength of the constant currents, expressed in rheobases, the points fall on a straight line (fig. 2, circulated nerve). This line represents the summation of the test shock and constant current and it passes through the origin where the test shock is just strong enough to evoke a half maximal alpha spike with zero constant current and through the point on the axis of constant currents where this is just strong enough to evoke a half maximal response with the test shock zero (fig. 2, circulated nerve). It is seen, therefore, that in the circulated

nerve over a range of constant currents from 50 to 100 per cent of rheobasic the test shock increment is directly proportional to the constant current decrement or that the converse is true. It is also evident (fig. 2, circulated nerve) that in the circulated nerve regardless of the duration of
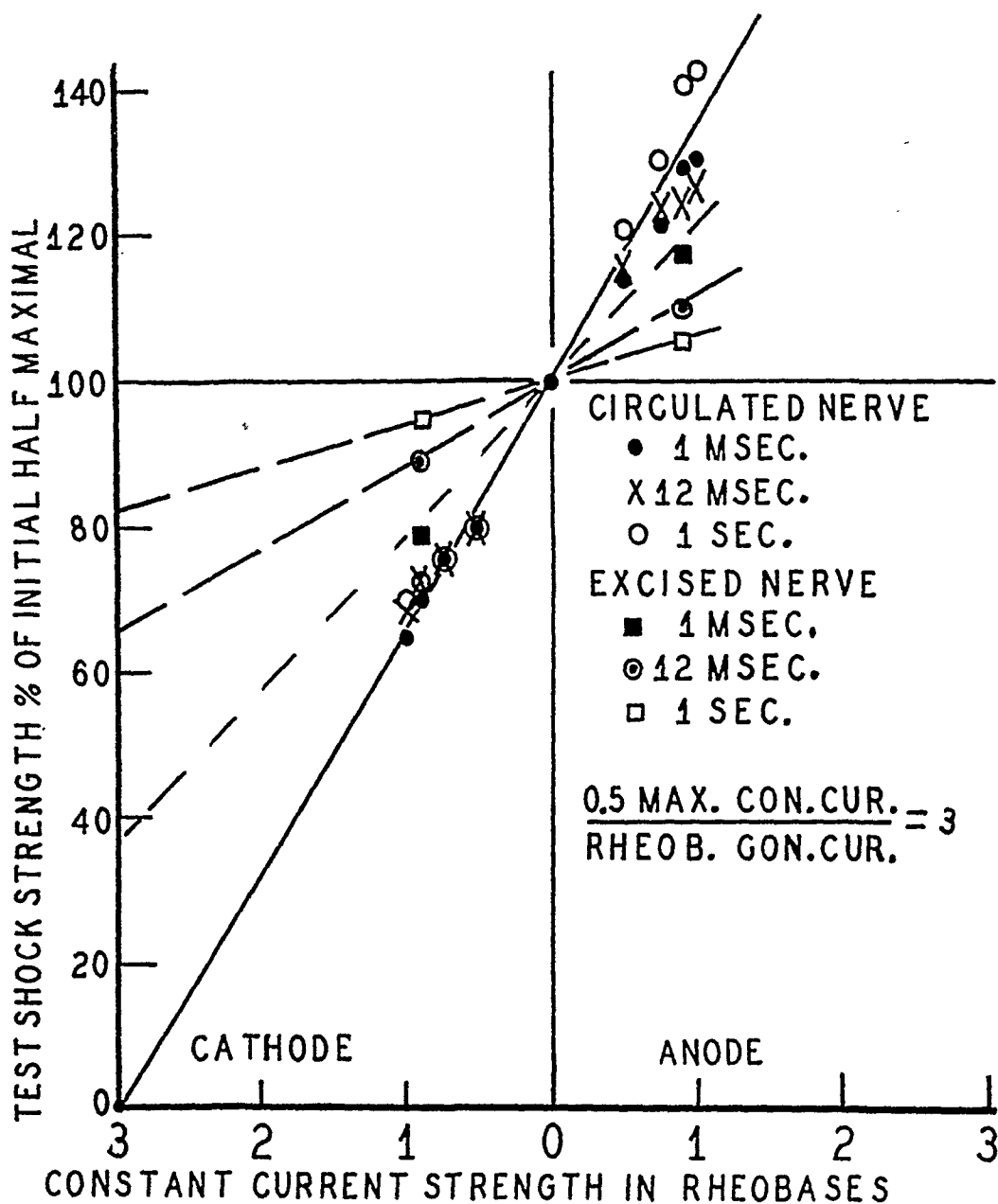
Fig. 2. Comparison of excised and circulated nerve.

Abscissae: Constant current strength at anode and cathode in rheobases.

Ordinates: Test shock strength. For the excised nerve, the point of intersection of the line through the test shock strength and the abscissa of ½ maximal constant current strength is a measure of the magnitude of the "decay of electrotonus."

1, 12, etc. is the time of application of the test shock after constant current make.

the constant current, up to one second, the points fall on the same straight line. There is, therefore, no evidence of a decay of the electrotonic state from its maximal value during the time intervals studied. The test shock is then a valid measure of the state of the electrotonus produced by constant currents of strengths varying from 50 to 100 per cent of rheobasic and of durations up to one second. Under conditions such that the electrotonic state rises and falls, the fall will be indicated by a rotation, toward the axis of 100 per cent test shock strength, of the line through the point having as its coördinates, test shock strength, and constant current strength used (fig. 2). When there is no rotation, that is when the experimental points fall on the line passing through the origin (100 per cent test shock strength, 0 constant current) and the half maximal constant current intercept, the decay of electrotonus, inhibition, adaptation or accommodation will be zero. When there is complete rotation, that is, to the axis of 100 per cent test shock strength, the decay of electrotonus will be complete or 100 per cent. Intermediate stages in the decay of electrotonus will be represented by points that fall between the above limits. If the line through these experimental points is projected back to the half maximal constant current intercept the magnitude of the decay of the electrotonic state is expressed as the percentage of a half maximal test shock that would sum with a constant current, which was originally half maximal in strength, to produce a half maximal response. In percentage the value is the same for other constant current strengths since it has been shown that the summation curve is essentially linear.

The responses of excised nerves to subrheobasic constant currents of varied durations may now be considered. Figure 2 (excised nerve) and tables 1 and 2 give details of the responses of excised nerve as the strength of the constant current and its duration are varied. As early as 1 msec. after the constant current make there is some decay of the electrotonic state in excised nerves as is evidenced by the failure of the electrotonus to reach its theoretically maximal value. From figure 1 the time required for the electrotonus to decay to one-half its maintained level (level at 12 msec.) can be measured. These times are at the cathode for 50 per cent of rheobasic constant current 2.7 msec., for 75 per cent of rheobasic current 2.3 msec., and for 90 per cent of rheobasic current 2.2 msec. At the anode the corresponding times are 2.8, 1.8 and 2.0 msec.

Study of figures 1 and 2 and tables 1 and 2 shows that these excised nerves exhibit excitability changes in the presence of subrheobasic constant currents which are comparable to those found by Erlanger and Blair (1932). The decay of electrotonus may be considered equivalent to the accommodation of Hill (1936) and is never complete. Neither are the mirror image excitability changes at the anode and cathode, required by the theories (Rashevsky, 1933; Monnier, 1934; Hill, 1936) to be found.

The cathodal decay of electrotonus is always greater than the anodal. In as much as electrotonus has been shown to be affected by temperature (Eichler, 1933), low temperatures (about 5°C.) decreasing it, the temperature has been maintained in all experiments at about 22°C. Also the

TABLE 1

Accommodation in excised nerve in relation to the constant current strength and time of current flow

CONSTANT CURRENT STRENGTH IN RHEOBASES	TIME AFTER CONSTANT CURRENT MAKE IN MSEC.	PER CENT ACCOMMODATION	
		Cathode	Anode
0.5	1.0	18	24
0.75	1.0	26	26
0.9	1.0	12	22
1.0	1.0	6	30
0.5	12.0	70	64
0.75	12.0	66	58
0.9	12.0	72	50
1.0	12.0	74	54
0.5	1,000.0	70	64
0.75	1,000.0	86	66
0.9	1,000.0	82	50
1.0	1,000.0	86	54

TABLE 2

Excised nerve showing the course of accommodation in relation to the duration of the current

This is a case of very marked accommodation

CONSTANT CURRENT STRENGTH OF RHEOBASES	TIME AFTER CONSTANT CURRENT MAKE IN MSEC.	PER CENT ACCOMMODATION	
		Cathode	Anode
0.8	1.0	0	10
0.92	1.0	4	10
0.8	8.0	68	44
0.92	8.0	83	44
0.8	1,000.0	100	72
0.92	1,000.0	100	100

time constant of accommodation (Solandt, 1936; Katz, 1936), and the blood calcium (De Boer, 1917), are markedly influenced by temperature. The maintenance of the temperature at a nearly constant level should materially reduce variations of these factors. Since the temperature

maintained was relatively high the development of both electrotonus and accommodation should be favored (Hill, 1936; Solandt, 1936).

It should be emphasized that the data thus far presented deals with the responses of excised nerves not washed or soaked in any physiological solution. These nerves show decay of electrotonus to a marked extent although subjected to no alteration of ionic environment other than that induced by removal to the moist chamber. The extent to which pH and factors dependent on it are altered is unknown and not subject to control. The differences in the responses of these nerves and the circulated ones
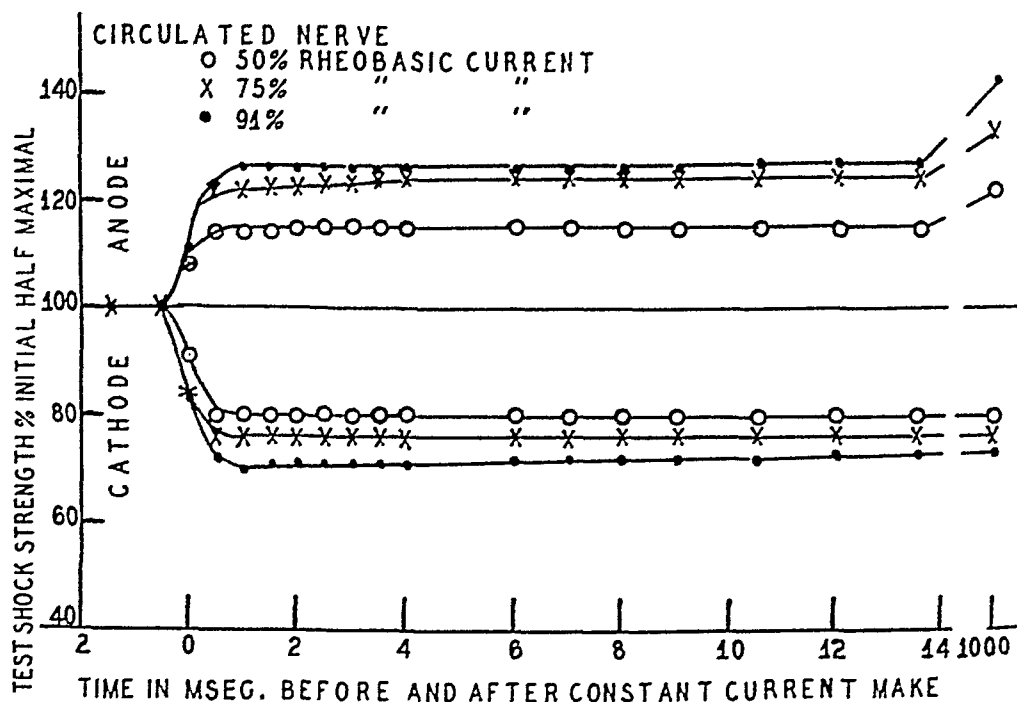
Fig. 3. Circulated nerve. Irritability at anode and cathode in the presence of 50 per cent, 75 per cent and 91 per cent of rheobasic constant currents. Compare with figure 1.

to be discussed next indicate that no small amount of change takes place in the nerve upon removal from the normal ionic medium maintained in the living animal.

Nerves provided with circulation. Studies were made on nerves in situ with the circulation maintained as nearly normal as was possible under experimental conditions. Although preparation for stimulating and recording led to some hemorrhage it was possible to maintain a reasonably good circulation when the operational procedures were kept at a minimum. The responses of circulated nerves to constant currents are shown in figures 2, 3 and 4. The details of the time course and magnitude of the

electrotonic state are shown in figure 3 which should be compared with figure 1 where similar data are presented for excised nerves. Figures 2, 3 and 4 demonstrate forcibly that there is essentially no decay of the electrotonic state, that is, no accommodation, in circulated nerves subjected to subrheobasic constant currents. Figure 3 shows clearly the marked depression of excitability developed at the anode when currents
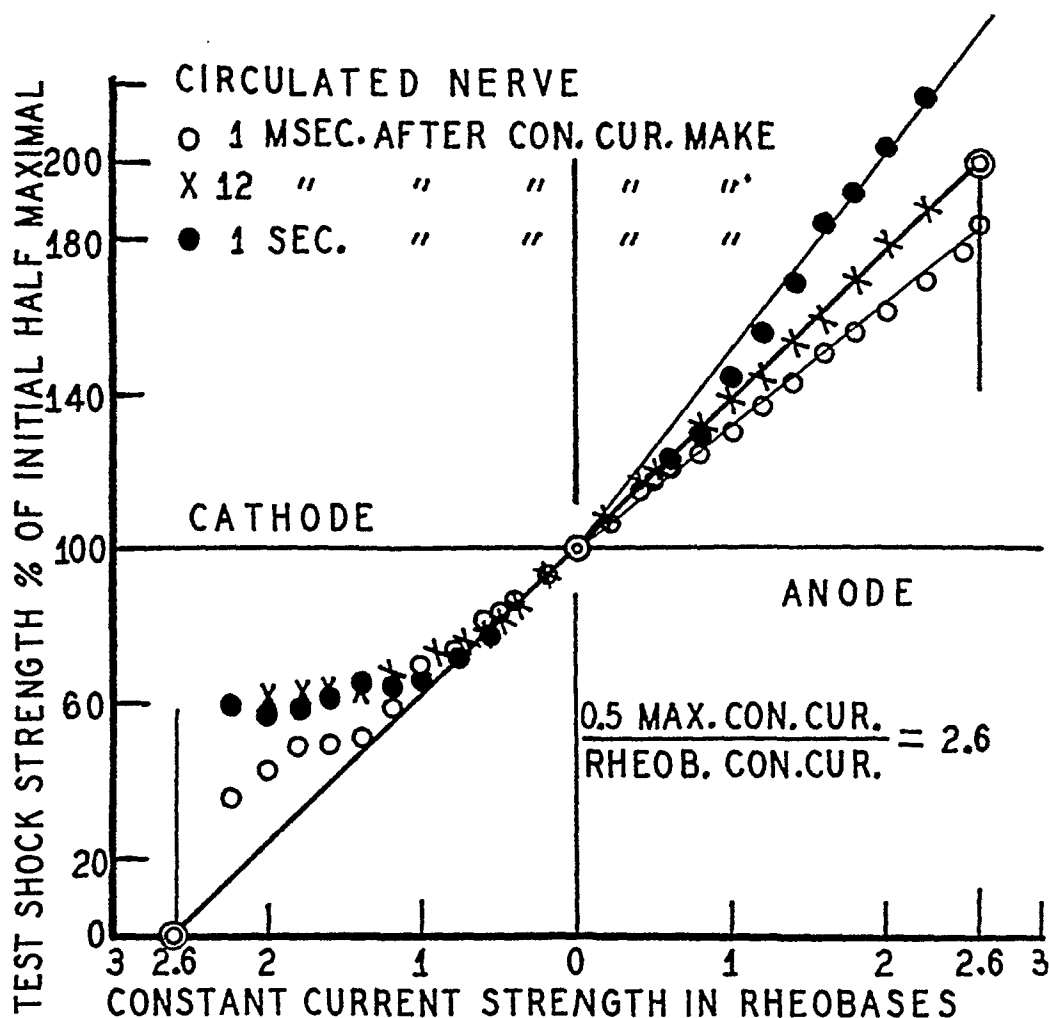
Fig. 4. Circulated nerve. Accommodation in circulated nerve. See text for detailed discussion.

of long duration are applied. Study of the figures will reveal other difference in the magnitude and time relations of the electrotonic states of the anode and cathode. An increase of the excitability at the cathode with currents of long duration sometimes occurs but this is never equal in magnitude to the anodal depression developed in the same nerve under the same conditions. Figure 4 gives data for currents increasing from subrheobasic to half maximal in strength. Compare the linear effects

produced at the anode by currents over this range with the effects of the same currents at the cathode.   The deviation from the linear relation at the cathode is to a large extent owing to the fact that the test stimulus falls in the refractory period of the nerve which follows its response to the constant current.   Such interference by the refractory period begins as soon as the constant current is of threshold strength for any fiber in the nerve trunk.   Since the response of these circulated nerves to the constant current is repetitive the interference persists as long as the constant current is applied.   Therefore, the test shock is increased in strength and the extent of the increase is largely determined by the point at which the shock falls in the refractory period following a response to the constant current.   No responses have been obtained from circulated nerves on the break of constant currents allowed to flow for one second or longer.   Figure 5 shows the excitability changes following the interruption of constant currents of 1 sec. duration and supplies a reason for the absence of the break response.   The anodal depression is seen to disappear rapidly at first and then much more slowly without, however, passing through a stage of increased excitability which could give rise to a break response. This is in marked contrast to the readily obtained break response of excised nerves and is in itself evidence for a negligible accommodation in circulated nerves.   Note the marked differences in the time courses of decay of the electrotonic state at the anode and cathode (fig. 5).

All the data obtained from circulated nerves indicated that when the circulation to the nerve was adequate no decay of the electrotonic state would occur.   The experiments of Liesse (1938a, b) point in the same direction.   In a few circulated preparations where no accommodation occurred (curves identical with fig. 3) ligation of the blood vessels to the nerve brought about a response identical with that of excised nerves (fig. 1) in 10 to 30 minutes.

Excised nerves soaked in various media.   The effects of soaking excised nerves (see figs. 1 and 2 and tables 1 and 2) in Ca^{++} and K^+ before applying the constant current are summarized in table 3.   Soaking the nerve in Ca^{++} rich Ringer brings about a response very like that of the circulated nerve except that the anodal depression is frequently much more pronounced.   The decay of electrotonus, accommodation, is much less than in unsoaked excised nerves.   Compare these effects of added Ca^{++} with the results of P. H. and M. Benoit (1937) on the removal of Ca^{++} by citrate injections in pithed frogs.   An opposite effect is obtained when the nerves are soaked in K^+ rich Ringer.   The magnitude of the decay of electrotonus, accommodation, is increased and the time to half accommodation is shortened.   In a freshly excised nerve the time to half accommodation was 3.8 msec. at the cathode and 3.7 msec. at the anode.   Soaking this nerve in excess K^+ reduced these times to 2.6 msec.
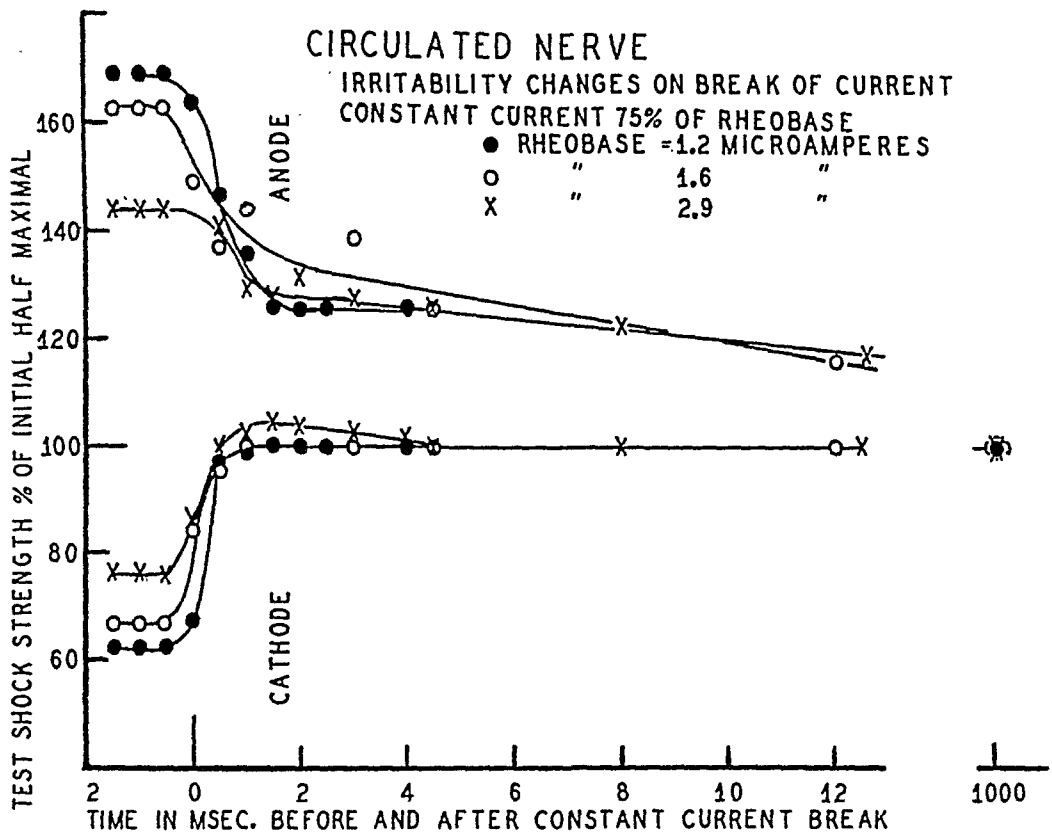
Fig. 5. Circulated nerve. Irritability changes on the break of a constant current of one second duration in circulated nerve.

TABLE 3

Comparison of the per cent accommodation in unsoaked excised nerve and in the same nerve after soaking in Ringer containing excess Ca^{++} or excess K^+

TIME AFTER CONSTANT CURRENT MAKE IN MSEC.	PER CENT ACCOMMODATION		PREVIOUS TREATMENT OF THE NERVE
	Cathode	Anode	
1.0	40	44	Excised nerve unsoaked
12.0	60	60	
1,000.0	76	60	
1.0	12	12	Same nerve after soaking in excess Ca^{++}
12.0	0	0	
1,000.0	−16	−120	
1.0	20	58	Nerve from opposite side after soaking in excess K^+
4.0	92	76	
12.0	92	82	
1,000.0	100	100	

at the cathode and 2.7 msec. at the anode.   The exact rôle of potassium is difficult to determine since Chweitzer (1935) and Bouman (1937) have found that it decreases catelectrotonus while Hill and Solandt have found that it decreased the time constant of accommodation.   It is not possible to assign values to these two effects from the data of the present experiments.

It is probable that the Ca^{++}/K^+ ratio is more important in the normal functioning of the nerve than the absolute amounts of either. Lehmann (1937a and 1937b) has found a close correlation between the pH and the Ca^{++}/K^+ ratio.   The pH was not controlled in these experiments and the extent to which alterations of pH may have altered the Ca^{++}/K^+ ratio is unknown.   The data obtained on the effects of Ca^{++} on the threshold for electrical stimulation are in agreement with the findings of Lehmann.   Neither Hill's normal Ringer, used by Solandt (1935), nor that of Kato (1934) maintained the nerve so that the electrotonic phenomena were the same as in the circulated nerve.   It is probable, therefore, that the circulation maintains the pH and the Ca^{++}/K^+ ratio about the nerve so that decay of electrotonus, accommodation, does not occur.   Similarly the removal of the nerve from the body to a moist chamber alters the pH and the Ca^{++}/K^+ ratio in such a way that accommodation is the usual response.

CONCLUSIONS.   In experiments performed on the frog's sciatic nerve the excitability of the alpha fibers, during the passage of subrheobasic constant currents, has been investigated by the test shock method (Erlanger and Blair, 1932a, b), using the half maximal alpha action potential as the index of the nerve's activity.   It has been shown that with a particular duration of the constant current there is a linear or nearly linear relation between the strength of the required test shock and the strength of the constant current (figs. 2 and 4).   In view of this relationship the variations of excitability are adequately measured by variations of the required test shock.   The excitability of the nerve at any moment as measured by the test shock strength may conveniently be expressed as percentage of the test shock strength which, when applied alone, gives a half maximal response.   The excitability so measured is proportional to (j-ε) of Rashevsky (1933, 1938) or to (U-V) of Hill (1936).

The alterations of excitability that follow the application of subrheobasic constant currents have been measured in excised and in circulated nerve. The excitability change attains a maximal level shortly after making the constant current and, in the excised nerve, returns toward the resting level as the duration of the current is prolonged (figs. 1 and 2 and tables 1 and 2).   This return toward the resting condition has been called the "decay of electrotonus" but it may equally well be considered as inhibition, adaptation or "accommodation" in the sense of Hill (1936).   The

magnitude of the decline of excitability is a measure of the amplitude of accommodation and the magnitude of the final level of the altered excitability is a measure of the maintained electrotonus. Since, in freshly excised nerves, there was always some maintained electrotonus and since there were striking differences in its magnitude at the anode and cathode the complete accommodation and the mirror image relations of the anode and cathode, demanded by the two factor theories (Rashevsky, Monnier, Hill), were not found.

In circulated nerves the electrotonic state was maintained throughout the time of constant current passage. Usually it increased in magnitude with long durations particularly at the anode. This indicated that in circulated nerve accommodation is zero. The repetitive response of circulated nerves to suprarheobasic constant currents and the absence of a break response support this concept. Since it can be concluded from these observations that accommodation is essentially a property of excised, uncirculated nerves the two factor theories of excitation, which can all be shown to be identical and to require 100 per cent accommodation, must be altered to include an added term. This term in I (equation 1) has always been made zero theoretically and occasionally experimentally (Hill, Solandt, Lucas), and has been justified by the liminal gradient experiments of von Kries (1884a, b) and Lucas (1907). Since the present experiments and those of Liesse (1938a, b) suggest that the liminal current gradient and accommodation are properties of excised nerve the two factor theories must now include the term in I in order to explain the responses of both excised and circulated nerve. Under these conditions Hill's methods for measuring the time constant of accommodation (λ) do not apply. These experiments, therefore, strongly suggest the need for the investigation of other aspects of nervous activity in the presence of an adequate circulation before the data obtained from excised nerve is used too freely in interpreting normal nervous function.

SUMMARY

The excitability of the alpha fibers in the frog's sciatic nerve during the passage of a subrheobasic constant current has been measured by determining the strength of a short test shock necessary to evoke half maximal alpha spike potentials. As found by other investigators, the alteration of excitability in excised nerves is maximum at about one millisecond after the application of a constant current and usually falls to a steady value other than zero. The decrease and the final value of excitability are measures of the amplitude of accommodation and electrotonus respectively.

Excised nerves not exposed to Ringer's solution show both accommodation and electrotonus. Ringer's solution containing excess Ca^{++}

reduces the amplitude of accommodation and may make it zero or negative, but excess K^+ increases the amplitude of accommodation and shortens the time to half accommodation.

Nerves with intact circulation in spinal animals show a negligible accommodation up to one second after application for currents 50 to 90 per cent of rheobasic. After the circulation is blocked or during anesthesia, the amplitude of accommodation becomes comparable to that of excised preparations.

In all preparations the effects at anode and cathode are not mirror images of each other. When present, accommodation is always less at the anode than at the cathode, and negative accommodation in circulated preparations and excised preparations subjected to excess $Ca++$ is more marked at the anode.

If other experiments also demonstrate that accommodation is negligible under nearly normal physiological conditions, it should be considered as an abnormal alteration of electrotonus, excitability or threshold which is characterized by an amplitude as well as a time constant. The two factor theories of excitation allow this viewpoint if the minimal gradient and allied phenomena may be classified as abnormal.

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THE REDUCTION OF THE BLOOD PRESSURE OF HYPERTENSIVE DOGS BY THE ADMINISTRATION OF RENAL EXTRACT[1,2]

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The pressor effects of renin can be diminished by the previous administration of an extract of the kidneys and such extracts also reduce the blood pressure of rats with hypertension caused by the removal of renal tissue (1). The purpose of the present study was to determine whether a similar effect could be induced in other types of renal hypertension. For this purpose dogs with hypertension due to renal ischemia (2) were employed. Such animals differ from the hypertensive rats previously studied in several respects: the amount of functioning renal tissue is greater, and the duration of the hypertension is longer in such dogs than in the rats; renal ischemia is present in the dogs but absent, according to the best available evidence (3), in the rats.

METHODS. Hypertension was induced in a series of dogs[3] by the application of clamps to the renal arteries according to the procedure described by Goldblatt, Lynch, Hanzal and Summerville (2). The mean blood pressure was measured directly on the unanesthetized animals by puncture of the femoral artery with a needle attached to a mercury manometer. In all instances numerous daily measurements were made before, during and after treatment.

Renal extract was purified and concentrated according to the procedures described elsewhere (4). A total of eight dogs were treated by intraperitoneal or intravenous injection of the concentrated renal extract, each animal receiving several injections during the period of one to four days and treatment then being withheld for one or more weeks. Most of these animals received several such courses of treatment. Ten animals

were also given renal extract either with the food or in most instances by stomach tube three to five times daily for a period of one day to two weeks, the blood pressure being measured one or more times per day during and after the course of therapy.

All the extracts used in the present work were first assayed on rats. As a preliminary unit we have utilized that amount of extract which will cause a reduction of 50, 35 and 25 mm. in rats having initial blood pressures of 200, 175 and 150 mm. of mercury, respectively. The amount of extract administered to each dog varied from 5 to 200 times this unit. In general, it was found that the dog requires about thirty to fifty times as much extract as does the rat in order to produce a comparable reduction in blood pressure. This is in accord with what one would anticipate from the relative surface areas of the two species.

RESULTS. *Parenteral therapy.* Each of the eight dogs which received renal extract by the intravenous or intraperitoneal route exhibited a decline in blood pressure. This appeared within 6 to 24 hours after instituting therapy and persisted for several days after treatment had been discontinued. The degree of decline in blood pressure varied markedly in different animals, the greatest diminution being approximately 60 mm. and the smallest 20 mm. In most instances the pressure began to increase within two to three days after the cessation of therapy, increased rapidly to a level somewhat less than that of the control period and then gradually rose during one or more weeks, the control level being finally reached in some animals but not in others.

In most instances the treated animals exhibited untoward symptoms. On several occasions severe collapse occurred but more frequently the animals displayed weakness, apathy and vomiting. Two of the animals treated parenterally displayed only the mildest toxic symptoms, but the declines in blood pressure were just as great in these animals (fig. 1A) as in those showing more striking toxic effects.

It is well known that all tissue extracts may contain toxic and depressor substances. The immediate decline in blood pressure occurring within a few hours after the administration of renal extract might justifiably be ascribed to such nonspecific substances and until the preparations can be further purified there can be no certainty as to whether the observed diminution in blood pressure was dependent on the administration of toxic agents or of some specific renal substance which is lacking in hypertensive animals. For these reasons the parenteral route of administration was abandoned when it was found that oral administration was efficacious in reducing the blood pressure, for it would seem that the disadvantage of having to use larger amounts of extract was more than offset by the reduced likelihood of obtaining non-specific depressor effects.

Oral therapy. Fourteen courses of treatment were given to ten dogs.
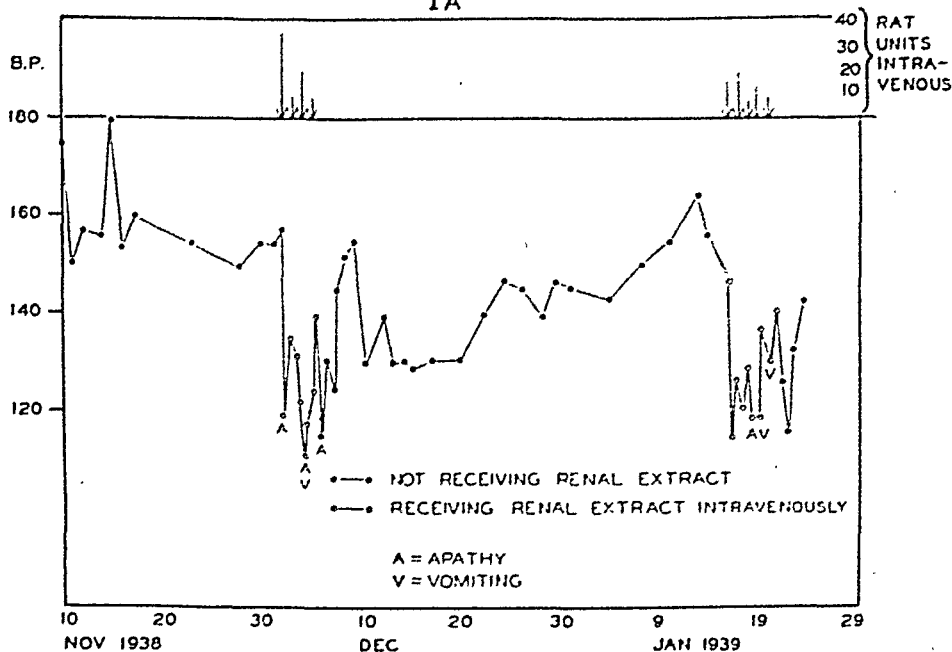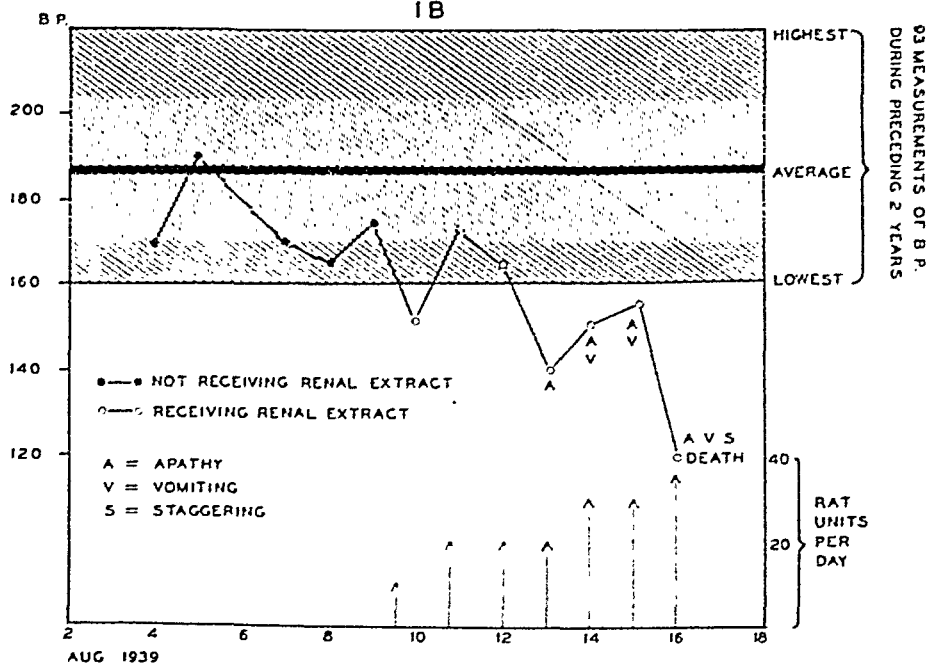
Fig. 1A. Intravenous injection of renal extract was followed by a decline in mean blood pressure of approximately 40 mm. After treatment ceased the blood pressure rose slowly and declined again when therapy was resumed. During treatment the animal vomited occasionally and was slightly apathetic.

Fig. 1B. Slight reduction of blood pressure following the oral administration of renal extract was accompanied by no symptoms. As the dose was increased and the pressure declined further uremia and death occurred.

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In ten instances a decline of blood pressure of 20 mm. or more below the average pre-treatment level was observed. In one experiment the blood pressure declined but so slightly (15 mm. of mercury) as to be of doubtful significance. In the remaining three instances no appreciable change in pressure occurred. These three animals received each a total dose of less than twenty units. The total dose in all of the other animals was fifty units or more.

We had hoped that oral therapy, if successful in reducing the blood pressure, would not be attended by toxic effects. However, such was not the case, administration of large doses of extract often inducing undesirable symptoms. The usual phenomena displayed were apathy and weakness. One of the ten dogs vomited frequently, five others vomited occasionally during treatment, and the remainder did not vomit. One of the dogs which displayed marked decline in blood pressure developed uremia and died. In two other animals which displayed a decline in blood pressure under treatment and well marked weakness and apathy, measurements of the non-protein nitrogen of the blood revealed minimal evidence of renal insufficiency. It was observed that those animals which had the highest initial blood pressures and hypertension of the longest duration, usually displayed the most marked symptoms of intoxication during treatment. Three dogs with mild hypertension exhibited no toxic symptoms at all either during or after treatment, in spite of a well marked diminution in blood pressure. However, the toxic symptoms were not entirely due to decline in blood pressure for two of the three animals which received minimal doses and had no decline in pressure also displayed mild untoward symptoms.

In figures 1B, 2A and 2B are reproduced typical experiments showing the changes in blood pressure following oral therapy with renal extracts. The control values cited on the figures before therapy were in the range of those observed for many weeks previous to the therapy and show the general range of unavoidable variation in the daily readings.

The dog of figure 1B promptly responded to the administration of extract with a reduction from an average of about 175 mm. to 150 mm. without any obvious detrimental effect on the animal. When the administration of the extract was continued and larger doses were used a further fall in blood pressure to the normal value of 120 mm. occurred on the seventh day of treatment. At this time manifestations of uremia were observed and the animal died 24 hours later.

In figure 2A is shown the result of an experiment in which the animal showed symptoms of apathy and vomiting when the blood pressure had declined from its original value of 190 to 163 mm. When therapy was discontinued and the pressure rose to 175 the symptoms disappeared
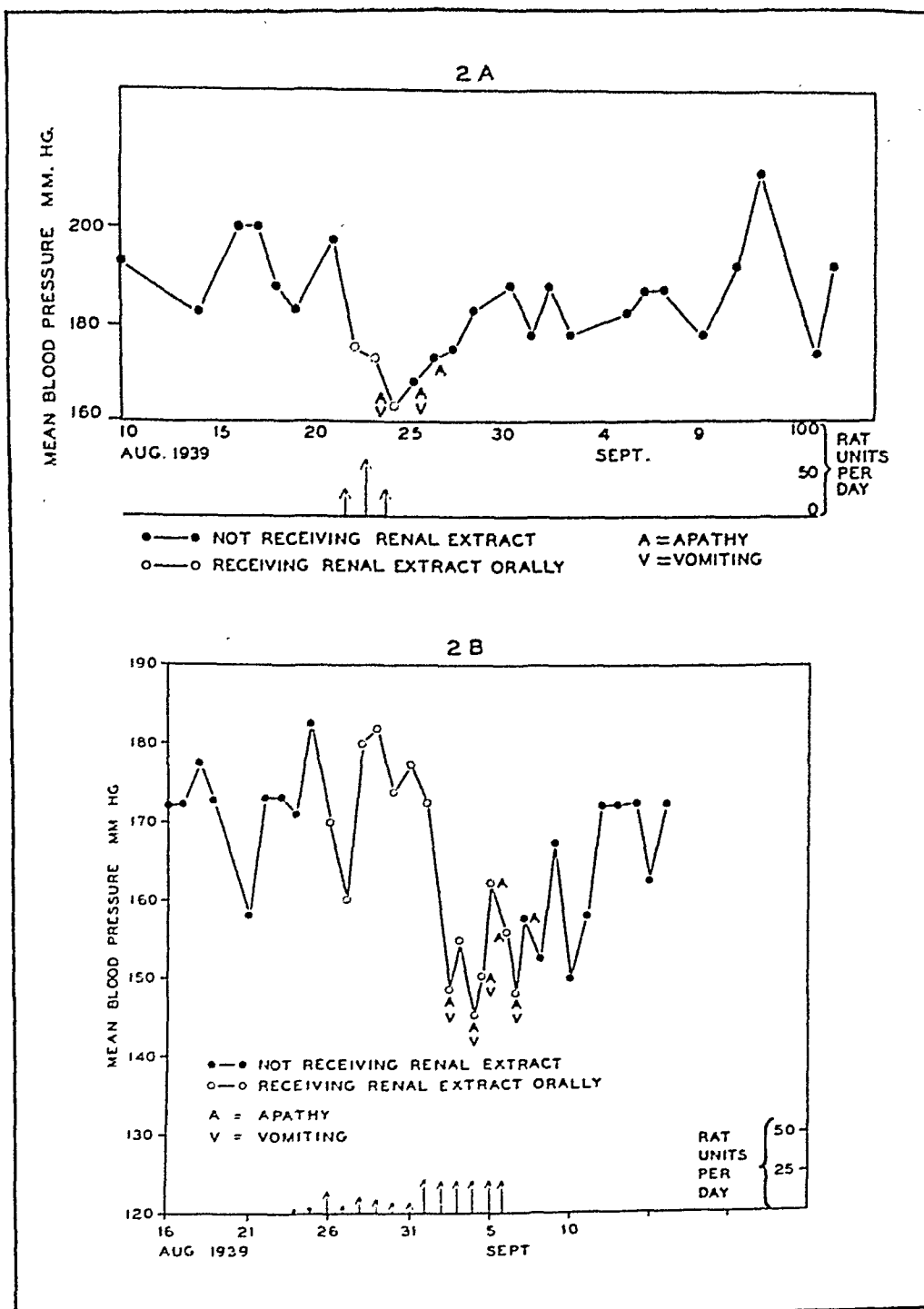
Fig. 2A. The administration of renal extract by mouth was followed by a prompt although slight decline in blood pressure and by weakness and vomiting.

Fig. 2B. Large doses of a relatively inactive extract produced no symptoms and no decline in blood pressure. When a more active extract was administered the blood pressure declined about thirty millimeters and mild untoward symptoms occurred. After treatment was discontinued the blood pressure gradually rose to the original level.

and the animal remained symptom-free on the following days, despite the reduction in blood pressure below the pre-treatment values.

Figure 2B reproduces the results obtained on an animal to which large doses of relatively inactive extracts (as assayed on rats) were first administered without any appreciable change in the blood pressure. The volume of extract administered and its content of contaminating substances present were essentially those present in the potent extracts. The fact that no symptoms were induced by this procedure would indicate that these are—in part—results of the decline in blood pressure rather than entirely due to any impurity present in the extract.

DISCUSSION. The observations which have been cited indicate clearly that the administration of properly prepared and concentrated extracts of normal renal tissue either parenterally or orally may cause a definite decline in blood pressure of dogs with hypertension due to renal ischemia. However, since the diminution obtained by the parenteral route of administration was accompanied by some abnormal symptoms in all of the animals and by severe illness in some of them, there is a definite possibility that the effects observed in this group of experiments may have been due to toxic and non-specific depressor substances. Such an objection is, however, less applicable to those experiments in which the renal extract was administered orally. Here again certain untoward manifestations sometimes occurred. There is, however, reason to believe that these undesirable symptoms were partly the result of the decline in blood pressure rather than entirely due to any toxic effect of the extracts.

In animals with highly elevated blood pressure any appreciable drop in this pressure must result in a deficient blood supply to the kidney, due to the presence of the clamp. A further factor to be considered is the generalized arteriolosclerosis which is known to follow long-standing experimental arterial hypertension. A subsequent reduction in blood pressure in such an animal would necessarily result in ischemia of the tissues. The effects of this ischemia in the nervous system and other vital organs would account for the untoward symptoms observed when the pressure was appreciably lowered in animals with long-standing hypertension of high degree.

One point merits especial emphasis. The phenomena described in the dog—namely, the decline in blood pressure to a normal level without untoward symptoms when the hypertension was mild, but severe symptoms and even death associated with the decline in pressure when the hypertension was more severe—are quite like those which we have already described in the rat (1). This similarity in response constitutes further evidence that the hypertension due to renal ischemia and that due to a deficiency of renal tissue are results of some common underlying renal disorder.

SUMMARY

A series of 18 hypertensive dogs were treated with renal extracts. The blood pressure of these animals was appreciably reduced, normal values being attained in some cases. Severe untoward symptoms accompanied this reduction in many cases. The bearing of these findings on certain aspects of the problem of renal hypertension has been discussed.

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THE EFFECT OF CASTRATION AND ANDROGEN THERAPY ON CREATINE AND CREATININE EXCRETION IN MONKEYS[1]

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The relation of creatine excretion to age and sex differences in man, and to species and sex differences in animals, is generally known. Children of both sexes and adult women excrete creatine, even while on a creatine-free diet, while adult men do not. The capacity to retain exogenous creatine is much greater in men than in women or children (Hunter, 1928).

In view of the above findings and those of Read (1921) and McNeal (1922), who have shown that eunuchs excrete creatine, interest has been focussed on the problem of whether a hormone or a lack of a hormone is responsible for the creatinuria. However, Tsun-Chee-Shen and Lin (1927) observed a creatinuria in but 1 of 9 eunuchs.

Kun and Peczenik (1932) have induced a creatinuria in rats when castrated post-puberally and which was subsequently suppressed by a urinary androgen (Proviron). Buhler (1933) has been successful in reducing the creatinuria occurring in alleged sexual hypofunction by the administration of androgen. Remen (1932) has shown that creatine retention is impaired in old age and castration. Retention can be improved by androgen or gonadotropic factor (Nitzescu and Gontzea, 1937).

Recently, Kenyon et al. (1938, 1940) have markedly reduced the creatinuria occurring in a eunuchoid, as a result of daily ingestion of creatine, by giving 25 mgm. testosterone propionate per day. In rats (Coffman and Koch, 1940) no creatinuria occurs after castration, nor is the capacity to retain exogenous creatine reduced below that of normal rats. The creatinuria, however, which is induced by feeding creatine, can be greatly reduced by the administration of testosterone propionate.

Koven, Beard (1939, 1940), Pizzolato and Beard (1939) have performed a long series of experiments on rats. They have observed a creatinuria

after castration in both males and females. Curiously enough they report that a creatinuria was also produced by injecting testosterone, estrogen, anterior pituitary extracts, cortin, saline and water.

Tsun-Chee-Shen (1927), Sandberg, Perla and Holly (1938) have failed to observe any change (in dogs and rats) in creatine excretion as a result of castration. Kochackian and Murlin (1935) did not observe a creatinuria in castrated dogs on a creatine-free diet, but did when the dogs were on a diet containing creatine.

EXPERIMENTAL. The experiments reported here are concerned with the relation of androgen and castration to creatine metabolism in the monkey. Nine male rhesus monkeys (Macaca mulatta) were used in this study, with each animal serving as its own control. They were kept in individual metabolism cages and on a diet practically free of creatine (consisting of fresh fruits and vegetables and a boiled egg thrice weekly). Twenty-four hour samples of urine were analyzed for creatinine and creatine according to the methods of Folin (1914) and modification of Benedict and Myers (1907) respectively. Each determination was done in duplicate; the creatinine values are expressed in milligrams and the creatine in milligrams creatinine.

The animals were castrated under ether anesthesia with sterile technique.

Creatine retention tests were performed in the following manner: 227 mgm. creatine hydrate (200 mgm. creatine), dissolved in sterile saline, were injected intraperitoneally. Two specimens of urine were collected, one after 24 hours and the other after 48 hours, and the total amount of creatine excreted was expressed in percentage excretion. On the average, two creatine retention tests were done for each procedure.

Testosterone propionate and estradiol benzoate were injected intramuscularly in sesame oil, and the human chorionic gonadotropin (PU.),[3] in sterile saline, was given subcutaneously.

Since glucose and acetone bodies interfere with the Jaffé reaction, the urine was tested qualitatively for these substances at various intervals, but in no case were these substances found.

Very careful records of the food intake were made during a considerable part of this study, but no correlation could be made between the creatine and creatinine values and the type and quantity of food, and no effect was observed after egg feeding.

Creatine excretion in immature monkeys. Immature monkeys normally excrete creatine as well as creatinine. Three of the four monkeys showed a creatinuria while the other excreted creatine for only two days during

the control period.   When given creatine retention tests, these animals did not retain more than 30 per cent and excreted the remainder.

The injection of testosterone propionate or human chorionic gonadotropin results in the abolition of the creatinuria, and increases the capacity to retain exogenous creatine (table 1).

Monkeys 1 and 2 (3.8 and 3.3 kilo) were both immature monkeys with inguinal testes.   Monkey 1 excreted during a control period of 13 days a daily average of 115 mgm. creatinine and 17 mgm. creatine; monkey 2 was creatine-free except for 2 days, and excreted 110 mgm. creatinine. However, the average for two retention tests on each animal showed that neither retained more than 31 per cent and excreted the remainder. Five milligrams of testosterone propionate were then administered to

TABLE 1

The effect of androgen treatment on immature monkeys

MONKEY	CONTROL PERIOD				INJECTION PERIOD			
	Days	Creatinine	Creatine	C.R.T. (excretion)	Days	Creatinine	Creatine	C.R.T. (excretion)
		mgm.	*mgm.*	*per cent*		*mgm.*	*mgm.*	*per cent*
1	13	115	17	70	15	122	3	33
	6	142	30					
2	13	110	0	71				
3	13	147	25		10	153	0	
	5	162	35					
4	8	236	38		7	216	0*	

* 400 r.u. Follutein on alternate days.

no. 1 for 15 days, resulting in a gradual abolition of the creatinuria.   The average for two retention tests resulted in 33 per cent excretion.

After cessation of androgen therapy, the creatinuria reappeared (33 mgm. per day) within 96 hours.   (Fig. 1 shows the daily excretion values for monkey 1.)

Monkey 3 (4.8 kilo) exhibited a creatinuria for 11 days during a 13 day control period; 147 mgm. per day of creatinine and 25 mgm. per day of creatine were excreted.   Two milligrams of testosterone propionate per day for 10 days abolished the creatinuria (except for 1 day) which subsequently returned within 48 hours after cessation of treatment (fig. 2).

Monkey 4 (6.5 kilo) excreted a daily average of 236 mgm. of creatinine and 38 mgm. of creatine during a control period of 8 days.   This animal was of adult size and had scrotal testes.   Since it showed a creatinuria,

one testis was removed for histological study. It resembled that of an immature monkey, having no sperm, few spermatocytes, and as far as could be determined, very few interstitial cells of Leydig. Four hundred rat units of human chorionic gonadotropin (Follutein) givin on alternate days caused a complete disappearance of the creatinuria within 72 hours. The remaining testis was then removed and the creatinuria set in again.

It was then attempted to see whether estrogen therapy had any effect in reducing the creatinuria which occurs in immature monkeys. Monkey 1 was injected with 100 r.u. per day of estradiol benzoate but there was no significant reduction in the creatine output. Creatine retention tests showed an average of 72 per cent excretion.

Creatine excretion in mature monkeys. Of 5 mature monkeys studied, 2 were entirely creatine-free during the control period; 1 had a creatinuria
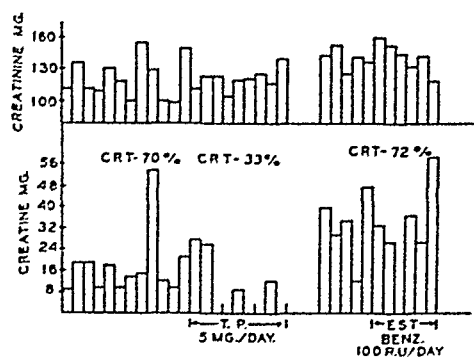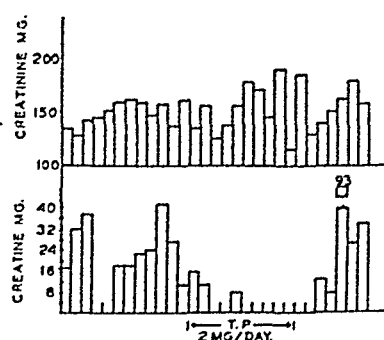
Fig. 1. Monkey 1. Each vertical bar represents a 24 hour sample. C.R.T., creatine retention test expressed as per cent excretion. *T.P.*, testosterone propionate. *Est. benz.*, estradiol benzoate.

Fig. 2. Monkey 3. Same symbols as in figure 1.

one-third of the time, while the others excreted creatine about one-half the time. All of the animals, however, reacted similarly to exogenous creatine by retaining most of it. After castration the creatinuria became more intense in all the animals except in those which excreted the most creatine during the control period.[4] The ability to retain exogenous creatine was now impaired.

The injection of 5 mgm. per day of testosterone propionate within 2 to 5 days completely abolished the creatinuria which ensued as a result of castration. The animals were now tested for their creatine retention

[4] It is interesting to note that monkey 7 and monkey 9 both had inguinal testes, which were small, aspermic and showed few interstitial cells of Leydig. These animals were castrated because of the fact that they showed excellent retention of exogenous creatine. Although no. 9 was not affected by castration, testosterone propionate abolished the creatinuria in both.

capacity and retained even more than during the control period (table 2). Although two of the animals were not affected by castration, the androgen completely abolished the creatinuria in all the monkeys.

TABLE 2

The effect of castration and androgen treatment on creatine and creatinine excretion in mature monkeys

MON-KEY	CONTROL PERIOD				CASTRATE PERIOD				INJECTION PERIOD			
	Days	Creati-nine	Crea-tine	C.R.T. (excre-tion)	Days	Creati-nine	Crea-tine	C.R.T. (excre-tion)	Days	Creati-nine	Crea-tine	C.R.T. (excre-tion)
		mgm.	*mgm.*	*per cent*		*mgm.*	*mgm.*	*per cent*		*mgm.*	*mgm.*	*per cent*
5	18	204	*	32	14	185	25	100	7	189	0†	15
					5	199	34		15	205	0	
					16	198	23	78	9	199	0	22
					8	219	72	100				
6	12	146	0	24	17	149	15	44	13	148	0	13
					10	160	19	52				
7	14	88	14	11	19	132	14	20	12	138	0	0
					9	146	42	77				
8	14	175	0	25	15	170	24	60	8	161	0	15
					8	177	27	44				
9	14	165	19	16	17	172	17	24	9	159	0	20
					6	199	18	13				

* Intermittent creatinuria.
† Does not include days when creatine values were declining.
C.R.T. = creatine retention test.

TABLE 3

The effect of estrogen treatment on creatine and creatinine excretion

MONKEY	DAYS	CREATININE	CREATINE	C.R.T. (EXCRETION)	DOSE
		mgm.	*mgm.*	*per cent*	*r.u. per day*
1	6	142	30	72	100
5	8	176	45		100
5	6	231 ·	38	79	200

After cessation of androgen therapy the creatinuria reappeared. Of the two monkeys in which there was little change in creatine excretion immediately after castration, one (no. 7) later developed an intensified creatinuria. During this second castrate period the animals (except no. 9) had an impaired capacity to retain exogenous creatine.

In figures 3 and 4 is the record of the daily output of creatinine and creatine for monkeys 5 and 6.

The above procedure of alternating injection periods with castrate periods was repeated three times on monkey 5, during a period extending over 9 months, with similar results each time. The administration of 100 and 200 r.u. of estradiol benzoate had no effect on the creatine excretion or creatine retention tests.

None of the procedures performed—castration, androgen, gonadotropic or estrogen therapy—had any significant effect upon the excretion of creatinine.
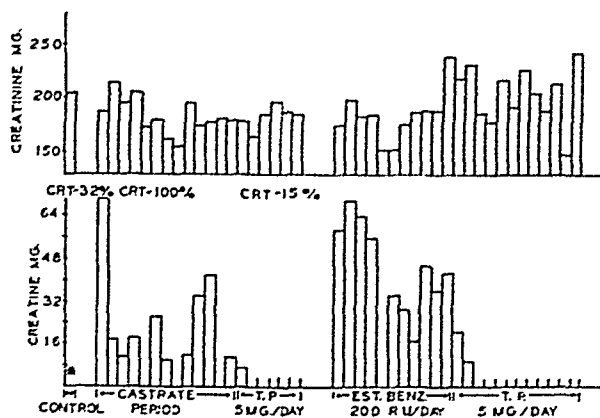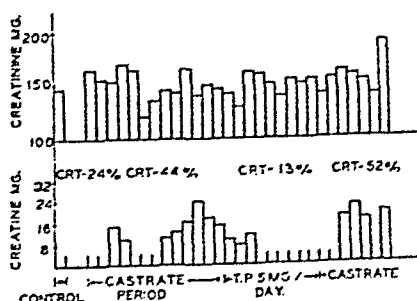
Fig. 3   Fig. 4

Fig. 3. Monkey 5.   *Intermittent creatinuria, control period is an average of 18 days.   Same symbols as figure 1.

Fig. 4. Monkey 6.   Control period is an average of 12 days.   Same symbols as in figure 1.

DISCUSSION. It appears clear from the results presented above that the creatinuria which usually occurs in young monkeys can be abolished by the administration of androgen. These monkeys do not tolerate exogenous creatine to any great extent and excrete most of the injected creatine. However, after androgen therapy the capacity to retain creatine is enhanced. Older monkeys may or may not exhibit a creatinuria, but when exogenous creatine is administered intraperitoneally most of it is retained. After castration a creatinuria appears, or is usually intensified if present during the control period. During this castrate period the animals usually do not retain creatine to as great an extent as previous to castration. Testosterone propionate injections result in the disappearance of the creatinuria in all cases, whereas estrogenic therapy, in the doses used, is ineffectual.

Immature or castrated monkeys do not store or metabolize creatine as well as do mature or androgen-treated animals. The reason that

children, women and eunuchs (even on a creatine-free diet) excrete creatine may be due to the fact that creatine formed from endogenous sources cannot be stored or metabolized as well as in the adult normal man.

In hyperthyroidism, muscular dystrophies, infectious diseases and many other conditions, an intense creatinuria occurs (Hunter, 1928). This may vary from 50 to 100 per cent of the creatinine values. However, the creatinuria of childhood or castration is of a much smaller magnitude, varying from 10 to 30 per cent of the creatinine values. About a month after the studies on one of the above monkeys were concluded, symptoms of tuberculosis were noted. The urinary creatinine averaged 155 mgm. per day while the creatine varied from 40 to 120 mgm. The injection of 5 mgm. of testosterone propionate daily had no effect upon the creatine excretion which continued to mount. The animal was killed and the clinical diagnosis of advanced pulmonary and generalized miliary tuberculosis was confirmed at autopsy. Thus, it would appear that the causative factors in these cases are different and the therapeutic agents effective in one may be ineffectual in the other.

It should be pointed out that impaired creatine retention occurs during hyperthyroidism, advanced muscular dystrophies and other conditions (Hunter, 1928; Milhorat and Wolff, 1938; Richardson and Shorr, 1936, and others).

Schrire and Zwarenstein (1934) have shown that in the rabbit, castration or ovariectomy results in an increased creatinine excretion which occurs after a latent period of months. However, some of the monkeys studied have been under observation for as long as 8 months after castration and yet no significant increase in creatinine excretion was noted. A human castrate has also been observed in the Squier Urological Clinic,[5] nine months after castration without treatment, and yet no significant increase in creatinine excretion occurred.

Recent work has indicated that the androgen may have an effect on general body economy as well as on the accessories. Papanicolaou and Falk (1938) have shown that injection of testosterone propionate will cause an hypertrophy of the temporal muscles of castrated male or female guinea pigs. Kenyon et al. (1939) found that androgen therapy would produce retention of Na, N, P, K, Cl, together with an increase in weight due to water held in association with salts and proteins retained. Hesser, Langworthy and Vest (1940) have reported increase in muscle work in myotonia atrophica cases with the administration of testosterone propionate.

Kenyon and his associates have discussed the general somatotropic influence of the androgen in man. Heretofore studies of the effects of androgen and estrogen have been concentrated on the sexual accessory

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[5] This patient was placed at my disposal through the courtesy of Dr. G. W. Fish.

organs, but in view of the fact that testosterone has an effect on Na, N, P, Cl, K, as well as water retention, creatine excretion, muscle mass and work, blood vascular system, as well as many subjective symptoms (Steinach, Kun and Peczenik, 1937), it seems possible that the androgen has an effect which is more general than considered previously. What the specific effects on body economy are, remain unknown at this time. However, in studying the metabolic effects, the differences between the physiological and toxic effects must be distinguished, since it is known that excessive dosages of androgen and estrogen may be toxic.

I should like to express my appreciation to Dr. E. T. Engle for his invaluable advice and encouragement during this study.

SUMMARY AND CONCLUSIONS

Young male monkeys usually excrete creatine, and show impaired retention of exogenous creatine.

Injection of testosterone propionate causes an abolition of creatinuria and increases the capacity to retain creatine.

Mature monkeys may or may not exhibit a creatinuria, but retain exogenous creatine.

Castration in mature monkeys results in an increased creatinuria and impaired creatine retention.

The injection of testosterone propionate abolishes the creatinuria of castration and restores the capacity to retain creatine.

Estradiol benzoate therapy is without effect on creatine excretion.

None of the procedures reported produced any effect on the level of creatinine excretion.

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THE DISTRIBUTION OF BLOOD PERFUSATES IN CAPILLARY CIRCULATION[1]

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Difficulties have been encountered in the perfusion of blood vessels with artificial solutions consisting of a mixture of salts as found in blood and a colloidal component to provide the necessary osmotic pressure. Unpredictable variations occur in the ability of such media to prevent the appearance of edema. Hitherto, most of the attempts to overcome this deficiency have been to increase the concentration of colloidal material or to change the nature of the colloid. Red cells have also been added to the artificial blood substitutes. The improvement noted was ascribed by Saslow (7) to the more adequate oxygenation provided by such solutions.

An investigation of this problem was undertaken by directly observing the capillary vessels in the frog through the microscope while the animal was being perfused. It was found, irrespective of whether the solutions were purely crystalloidal or contained colloidal components, that an active flow of the perfusate occurred only in the a-v capillaries, no circulation being apparent in the numerous true capillaries. Because of this, the use of solutions colored with Evans blue afforded a remarkably good demonstration of the a-v capillaries in contrast to the true capillaries. The existence of two types of capillaries in numerous tissues has been emphasized in a previous publication (9). An important distinction between the two is effected by the different way in which the vessels leave the parent trunk. The a-v capillaries are those vessels which are direct continuations of the arteriole and constitute a relatively small portion of the capillary bed. The a-v capillaries are of considerable length and are not to be confused with extremely short anastomoses between arteries and veins, which are often referred to as A-V-A vessels. Coming off almost at right angles from this continuous trunk are large numbers of true capillaries. The a-v capillaries, being direct extensions of the arterioles to the venules, serve as bridging channels between them. The numerous true capillary offshoots, by virtue of their position, do not lie in the direct path of flow of the perfusing solution.

Tests were made to ascertain the part played by the formed elements in the distribution of the perfusate. For this purpose, particulate matter, either as a suspension of carbon or of red cells, was added to the various perfusion media. The resulting capillary circulation, regardless of the type of particulate matter employed, was a decided improvement over the restricted circulation previously obtained. Within a short time the perfusate was seen to circulate through all of the capillary vessels and the appearance of edema was considerably delayed. Since the proper distribution of the perfusion fluid is of prime importance, the factors that enhance this feature were studied, with emphasis on the rôle of formed elements.

Zweifach (10) has found that marked differences occur in the capillary pattern of various tissues. Krogh (5), Saslow (7) and Drinker (3) have used the capillary vessels of the frog's web for perfusion studies. However, the capillary bed of the web belongs to the skin type of vessels and is atypical, consisting of a rich meshwork of extremely short anastomosing channels. The capillaries of the web are further unsuited since, as Krogh (6) and Conklin (2) have shown, they are abnormally permeable to proteins. The vessels of the mesentery and the tongue of the frog are more satisfactory for perfusion studies because their capillary pattern is representative of that found in connective tissues and muscle in general. Furthermore, the degree of permeability of the capillaries in the tongue and mesentery is directly comparable to that of similar vessels in other tissues.

For these reasons, the studies described in this paper were restricted to direct observations made on the capillary circulation in the mesentery and tongue of the frog while the animal was being perfused. Details of the perfusion technique employed are described in an earlier publication (1). The colloidal materials added to the Ringer solutions were 0.5 per cent ash-free gelatin (Eastman Kodak Co.) and 3 per cent gum acacia (E. Lilly). Evans blue, because of its poor diffusibility through the capillary membrane, has been used in blood volume studies by Gregersen and Gibson (4). Particle-free solutions were colored with Evans blue in order to follow the circulation of the perfusate more readily. The perfusion fluid was introduced by cannulating the aorta, the effluent fluid escaping through the cut end of the ventricle.

A. *Restricted circulation with solutions lacking formed elements.* Colored Ringer solutions buffered at pH 7.5 were employed first. When perfused by way of the aorta they readily appeared at the point of venous outflow. The perfusion solution was seen to enter the a-v capillaries immediately and washed out all the blood cells from these vessels. The perfusate did not enter the true capillaries.

Before starting the perfusion, a true capillary, that left the a-v capillary and rejoined it within the field of the low power objective, was selected for observation. As soon as the blood had been washed out of the a-v

capillaries, the blood cells inside the true capillaries were sucked into the a-v current from the two ends of the vessel. This continued until the true capillaries were completely emptied of blood cells. The suction-effect, by virtue of which material was continuously being removed from both ends of the true capillaries and draining into the a-v capillaries, seems to have arisen as a result of the rapid flow of the perfusate past the orifices in the a-v wall leading to the side capillaries. The disposition of the true capillaries, which come off almost at right angles to the parent trunk, favors the development of such suction forces, especially when a rapid flow is found only in the a-v capillaries.

A second series of Ringer solutions was prepared by adding mannitol, a relatively impermeable inert sugar, in an amount calculated to maintain proper osmotic balance. The circulation of this solution, likewise, was found to remain confined to the a-v capillaries.

A third series of media consisted of Ringer solutions combined with ash-free gelatin or with gum acacia. These perfusates were found to be more efficient in reaching almost all of the a-v capillaries. None of the colored perfusion fluid flowed through the more numerous true capillaries. When a higher concentration of colloidal material was used, the viscosity of the solutions (especially in cold blooded forms where the perfusate was kept at room temperature) severely slowed the flow and limited its distribution more radically than before.

When the Ringer-gelatin solution was perfused at a low pressure (15 to 20 mm. Hg), a flow existed only in a small number of a-v capillary channels. By keeping the rate of flow constant at this level, a steady state developed in which the true capillaries contained no active flow but remained open. These static true capillaries remained patent apparently as a result of fluid seeping into them from the tissues and being drawn at a slower rate into the a-v channels. When the pressure was increased, the streaming of the perfusate through the a-v vessels was accelerated. The true capillaries then gradually narrowed down over a period of 3 to 4 minutes and became completely collapsed along their mid-regions. The factor effecting this narrowing appeared to be the augmented suction-effect produced by the rapid flow through the a-v capillaries.

The true capillaries did not collapse during perfusions in which crystalloidal solutions were used, presumably because considerable fluid was diffusing into them from the surrounding tissues. An active flow was not observed in all the a-v capillaries, but only in those which took the most direct course to the venous vessels. This restricted circulation persisted even when the perfusion pressure was increased from the normal level of 30 mm. to as high as 75 mm. Hg.

These experiments showed that the beneficial action of colloidal matter resides in part in its ability to promote an active circulation of the per-

fusate through a much larger number of a-v capillaries than occurred with crystalloidal perfusates. The superior distribution of colloidal mixtures over that of mannitol-Ringer solutions, may be due to the fact that the viscosity of the colloidal perfusates more nearly approximates that of blood. In tissues where the a-v type of capillary predominates (tongue), such solutions were almost completely adequate in filling the capillary bed.

B. *Rôle of particulate matter in distribution.* A suspension of fine carbon particles or of avian red cells was added to Ringer-gelatin and Ringer-acacia solutions. When the animal was perfused with these solutions, the circulating fluid not only coursed through the a-v capillaries, but filled the entire capillary bed so that all parts of the tissue were simultaneously exposed to the action of the perfusate. The following is a description of the changes that occurred during the period in which the circulation of the perfusate became wider in scope to include the true capillaries.

When the particle-free colloidal solution had been washed out of the a-v capillaries by a carbon-containing perfusate, peculiar swirls of carbon were seen to develop at the orifices in the a-v wall leading into the true capillaries. These capillary offshoots are given off from the a-v parent trunk at a sharp angle and with a marked backward twist. Similar characteristic swirls appeared when suspensions of red cells were used. In addition, these were accompanied by the lodging of red cells against the angular projections of the wall at the point of capillary exit. The trapped cells wavered back and forth until the axial current was suddenly deflected into the side channels. The flow in the capillary side channels was sporadic in character, with the red cells being distorted and slowly forced through the narrowed portions of the vessels. The movement of this column of cells at first was pulsatile, the red cells being drawn back and forth through the constricted vessel. This was accompanied by a slow opening of that portion of the vessel with which the red cells made direct contact, until a continuous flow through the capillary was established. The number of side channels into which the flow was deflected varied with the perfusion pressure employed. At comparatively low pressures (15 to 20 mm. Hg), the flow through the true capillaries was sporadic. When the pressure was raised to 35 mm. Hg, the perfusate was carried into the majority of available channels.

This deflection of flow was repeatedly produced in the same preparation by the alternate use of particle-free and particle-containing solutions. At the commencement of perfusions with particle-free solutions, the flow became confined to the a-v channels as soon as the blood of the host had been washed out. Within 30 seconds after changing to a carbon containing solution, the perfusate gained access to all the capillary vessels. Carbon tended to stick to the capillary walls, especially along the intercellular cement, and often clogged some of the vessels. Red cells were never ob-

served to stick to the wall or to one another and were more efficient. For these experiments it was not necessary to use the cells of the host. Rooster red cells suspended in frog Ringer-gelatin solutions gave a perfectly normal picture, with the perfusate streaming through all of the capillaries.

From the above data it can be seen that the presence of the suspended material was responsible for the setting up of mechanical disturbances at the points along the a-v wall where capillary offshoots emanate and thereby routed the perfusate into all of the capillary channels.

C. *Particulate matter in the control of edema.* When particle-free Ringer or Ringer-mannitol solutions were used for perfusion, pronounced edema occurred within 10 to 15 minutes. Colloidal Ringer mixtures, lacking formed elements in suspension, were capable of maintaining normal fluid balance for 30 to 40 minutes. As previously indicated, colloidal perfusates maintain a more adequate capillary circulation. This probably is an important factor in the delayed edema obtained with colloidal solutions as compared with its rapid appearance with isosmotic Ringer-mannitol mixtures. Ringer-mannitol solutions left the a-v capillaries at an extremely rapid rate as compared to Ringer-gelatin mixtures. It is significant that the colloidal perfusates coated the inner surface of the capillaries and thereby not only rendered the wall relatively less permeable to fluid loss, but also curtailed the passage of the colloidal component of the perfusate into the tissues. Ringer-mannitol solutions do not exhibit such coating properties. The streaming of crystalloidal perfusate through the a-v capillaries was accompanied by a washing off of the coating of the blood proteins of the host, leaving the vessel wall in an excessively porous state. The effectiveness of both solutions, however, is considerably diminished because of their poor distribution, the perfusate coursing through only a small fraction of the available channels.

A suspension of carbon in Ringer-gelatin delayed the appearance of edema for over 110 minutes. Red cell suspensions were somewhat more efficient in this respect, delaying the onset of edema for more than 180 minutes.

The greater effectiveness of solutions containing particulate matter over those which are particle-free depends upon at least two important properties. First, the perfusates bring about a more normal type of circulation throughout all the vessels of the capillary bed. Secondly, the presence of the particles increases the oxygenation of the solution. Warburg (8) has shown that suspensions of carbon particles have considerably more oxygen than ordinary solutions because of the increased surface provided for the adsorption of oxygen. The presence of red cells also markedly increases the oxygen carrying capacity of the solution. These two factors acting together would tend to diminish anoxemic effects and insure a more adequate removal of metabolic products from all parts of the tissue.

Solutions lacking suspended material prevent edema only for a short period of time. The temporary lack of edema is in large part due to the limited filtration surface exposed to the perfusate, since only a fraction of the total capillary vessels are actually perfused by the circulating fluid. This situation results in general asphyxia and the accumulation of metabolites in most of the tissue. An increase in the permeability of the capillary wall follows, causing the onset of edema.

D. *Rôle of particulate matter in plugging.* In addition to its rôle in the distribution of the perfusate, particulate matter was found to perform another important function. Chambers and Zweifach (1) have shown that temporary porous spots appear in the capillary wall and can be increased or decreased by variations in pH and calcium content of the perfusate. In the present study, when the perfusion was carried out for longer than two hours, the capillaries became increasingly leaky. The increased outward diffusion of fluid was accompanied by a flattening of the particulate matter against the capillary wall over these points. This phenomenon, especially when red cells were used, acted to reinforce the weakened portions of the capillary wall and curtailed seepage through actual leaks by forcing the extremely plastic red cells into them. Stasis of red cells also played a part in preventing excess loss of fluid. The cells quickly filled the abnormally leaky vessels as the fluid diffused out of them. The resulting mass of cells clogged the vessel, removing it from the active circulation, and thereby functioned as an additional safeguard against excessive loss of fluid.

E. *Mechanism of collapse and closure of capillaries.* As has been noted above, the true capillaries frequently became narrowed, even to the extent of complete occlusion, in experiments with particle-free solutions where the circulation of the perfusate was limited to the a-v capillaries. An attempt is made here to compare these results with similar phenomena in animals with an intact blood circulation.

Perfusion data. The arrangement whereby the true capillaries come off almost at right angles to the a-v vessels exposes them to suction effects arising from the continuous flow in the a-v capillary. This was conclusively demonstrated when the blood of the host was washed out by perfusing the vessels with Ringer-gelatin solutions containing Evans blue. Despite the fact that none of the colored perfusate coursed through the true capillaries, the blood cells contained in them were rapidly drawn into the a-v current from both the arterial and venous ends of the true capillary. The blood cells moved in opposite directions towards both ends of the true capillary. Such a movement could only be brought about by a suction simultaneously acting on both the exit and entrance of the true capillaries.

During the perfusions with Ringer or Ringer-mannitol solutions, fluid was rapidly lost from the a-v capillaries, resulting in pronounced edema

within 10 minutes. In this case, the true capillaries, despite the absence of an active flow in them, always remained open. With Ringer-gelatin or Ringer-acacia perfusates, the loss of fluid was considerably diminished, as evidenced by the absence of edema during the first 30 to 40 minutes of perfusion. When the perfusion pressure was kept low (15 to 20 mm. Hg), the true capillaries remained patent throughout the experiment. However, a gradual collapse of the true capillaries occurred by raising the perfusion pressure to 35 mm. Hg. During this period, the a-v rate of flow was considerably increased and concurrently there was a corresponding diminution in the caliber of the true capillaries. The experimental findings suggest that the collapse of these vessels was a direct result of the increased suction forces created by the more rapid streaming through the a-v capillaries. The closure was first evident along the mid-regions of the true capillaries and progressively spread in both directions towards the arterial and venous ends of the vessel. Such capillaries remained closed throughout the remainder of the experiment despite the appearance of edema after 40 minutes of perfusion.

The differences in caliber of the true capillaries obtained with crystalloidal and colloidal perfusates, despite the complete absence of flow in these vessels, can be explained on the following basis. The degree of narrowing appears to be a resultant between the rate of infiltration of fluid into the true capillaries from the tissues and the removal of fluid from them by suction forces arising in the a-v capillaries. The rapid loss of fluid with crystalloidal perfusates provides an excess of tissue fluid. The true capillaries therefore are continuously being supplied with fluid seeping in from the tissues. In colloidal-Ringer perfusions, the rate of fluid loss is minimal during the initial 30 to 40 minutes. Fluid slowly diffuses into the static true capillaries for only a short time, no excess being available in the tissues. The continued suction then acts to effect a narrowing of the true capillaries, which is evident within 15 to 20 minutes.

It should be emphasized that no active closure of the true capillaries was observed. The capillaries appeared to be affected passively by the flow coursing through them and by the creation of suction forces in the a-v vessels. Opening of the true capillaries was never observed during the period when no flow circulated through them. Likewise, a collapse of the true capillaries occurred only when they were devoid of flow concurrent with the existence of an active circulation in the a-v capillaries.

The use of perfusates containing particulate matter brought about a complete reparation of this situation. When low perfusion pressures (15 to 20 mm. Hg) were used, the circulation was most active in the a-v capillaries and sporadic or non-existent in the true capillaries. By gradually increasing the pressure to 30 mm. Hg, an active streaming was obtained in all the capillary vessels. The initial effect was an increased rate of flow

through the a-v channels. A maximum rate of flow is reached, above which a further increase in pressure acted to distend the vessel. As the a-v capillary widens, the orifice leading into the capillary offshoots became more patent. The higher pressure in the a-v capillary also tended to produce a more rapid filtration of fluid into the tissues. When the perfusate contained red cells, the loss of fluid led to a closer packing of the red cells, especially on the venous side. The red cells were no longer solely confined to the central, axial a-v current but were dispersed throughout the vessel. Eddies appeared where the true capillary offshoots branched out and were effective in deflecting the circulation into these side channels. Carbon suspensions effected a partial opening of the true capillaries. Red cells, on the other hand, were forcibly squeezed into the collapsed channels and thereby distended them to a point where the cells coursed freely through the capillary.

Intact blood circulation. Similar observations to the above were made in tissues with an intact blood circulation. In resting tissues, a continuous flow exists only in the a-v vessels, the true capillaries remaining relatively static and often extremely narrowed. Following mild stroking of the tissue with a microneedle, the arteriolar flow to the capillaries in the area increases. The initial effect of this was a more intense flow through the a-v capillaries. Ten to fifteen seconds later, a closer packing of the circulating blood cells can be noted in the distal portions of the a-v capillaries and the flow began to slow down. This is evidently brought about in part by the added loss of fluid resulting from the increased pressure. The narrowed true capillaries can be seen to open slightly. Eddies appeared in the a-v capillary at the points where the side branches are given off and soon the flow was deflected into these capillaries. Following a variable period, depending upon the intensity of the irritation, the arteriolar flow diminished. The flow in the true capillaries became sporadic and gradually ceased. With the flow again limited to the a-v capillaries, conditions for the reëstablishment of the suction forces at both ends of the true capillaries are present. The contents of the vessel drain into the a-v capillaries and the true capillaries become gradually narrowed.

SUMMARY

Solutions designed to replace the blood as a perfusion medium to be completely effective must not only contain appropriate chemical constituents but also a suspension of formed elements, such as carbon or red cells. The beneficial action of the particulate matter lies, in part, in its ability to bring about a maximum distribution of the perfusate through all of the vessels of the capillary bed. In the absence of red cells or carbon, the flow is restricted to the a-v capillaries. The addition of suspended particles diverts the flow into the true capillaries which are offshoots of the a-v

vessels.   Particulate matter, especially red cells, is effective also because it plugs up leaks in the walls of the vessels.   Stasis in abnormally leaky vessels is an additional factor which acts to prevent further fluid loss by clogging these vessels and cutting them off from the active circulation.

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THE PROLONGED ACTION OF ACIDIFIED SOLUTION OF PROTAMINE ZINC INSULIN

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In an earlier paper (1) from this laboratory, it was pointed out that when an acidified solution of insulin is subcutaneously administered it passes through various stages of hydrogen ion concentration until the reaction at the site of injection is in equilibrium with that of the tissue fluid, whereupon absorption takes place. On the basis of this theory, the question arises as to whether or not a clear aqueous solution of protamine zinc insulin would possess a prolonged hypoglycemic effect.

The prolongation of hypoglycemia following the administration of an insulin preparation to which protamine had been added in a phosphate buffered solution at about pH 7.3 was first reported by Hagedorn and his collaborators (2). Later Scott and Fisher (3) and Fisher and Scott (4) demonstrated that the addition of about 2 mgm. of zinc per 1000 units to such a protamine insulin preparation considerably prolonged its hypoglycemic effect. Since that time the addition of about 0.2 to 0.25 mgm. zinc per 100 units of protamine insulin has been a standard procedure.

This investigation is concerned with the preparation of a clear solution of protamine zinc insulin possessing a prolonged hypoglycemic action and a study of its effect on fasting rabbits.

GENERAL CONSIDERATIONS. Throughout this investigation a recrystallized preparation of insulin made in this laboratory was used. It assayed 22 international units per 1 mgm. The ash content did not exceed one per cent and its zinc content was about 0.3 mgm. per 1000 units.

Stock solution. A stock solution was prepared by dissolving 364 mgm. of insulin crystals in water to which had been added a few drops of one normal lactic acid. This solution was next transferred quantitatively to a 100 cc. volumetric flask and before diluting it to the 100 cc. mark 1.6 cc. glycerol and 0.1 cc. tricresol were added. The acidity of the solution was adjusted to about pH 3.0 (using the glass electrode). This stock solution contains 80 international units per 1 cc.

The following procedure is illustrative of the method employed for the

preparation of clear acidified solutions of protamine zinc insulin. Fifty cubic centimeters of the stock preparation containing 4000 units are transferred to an Erlenmeyer flask immersed in a water bath at a temperature of about 50°C. While stirring 200 mgm. of protamine are introduced followed by 7 mgm. of zinc as zinc chloride and 2 grams of sodium acid phosphate. The mixture becomes turbid at first but it clears up as the temperature reaches 45° to 50°C. The reaction of the mixture is adjusted exactly to pH 3.0 and the volume is increased to 100 cc. using a diluent containing distilled water, 1.6 per cent glycerol and 0.1 per cent tricresol. This mixture of an acidified solution of protamine zinc insulin consists of 5 mgm. of protamine, 0.2 mgm. zinc per 100 units of insulin in a 2 per cent phosphate buffer solution. Henceforth it shall be referred to as "clear solution of protamine zinc insulin."

In preparing dilutions for experimental work, the diluent unless otherwise specified consisted of a 2 per cent sodium acid phosphate containing glycerol and tricresol in the above mentioned ratios at an acidity of pH 3.0. In dilutions free from phosphate the addition of sodium acid phosphate was omitted.

Injections. All parenteral administrations were of deep subcutaneous injections. For dilutions containing 10 units per 1 cc. or less, a 1 cc. tuberculin syringe was used and the amount administered was proportional to bodyweight. However, when it became desirable to use a higher concentration of insulin such as 40 units per 1 cc. a micro syringe of 0.25 cc. capacity was employed.

Throughout this investigation the switch-over technique was used. The animals were divided into two equal groups. One group received one preparation and the other received another. The following week the order was reversed.

Rabbits that had been fasted for 24 hours were used. Blood was withdrawn from the marginal ear vein at the interval of time specified in each experiment. Sahyun's method (5) for blood sugar determination was employed.

EXPERIMENTAL. *1. The effect of dilutions.* In this experiment a comparison was made between the effects of each of the following preparations on the blood sugar of fasting rabbits:

A. A crystalline insulin preparation containing 5 units per 1 cc. *B.* A similar preparation of 5 units per 1 cc. of crystalline insulin to which had been added 2 mgm. zinc per 1000 units, and *C*, a solution containing 40 units per 1 cc. with 2 mgm. zinc per 1000 units. Twenty rabbits were used for each test and each animal received 2 units of insulin. The switchover technique was employed. Samples of blood for sugar determination were withdrawn at 0, 1.5, 3, 4 and 5 hours respectively. The results of this experiment are found in figure 1.

2. Herein are shown the differences between the effects of *A,* a clear buffered solution of protamine zinc insulin; *B,* a precipitated protamine zinc insulin (the marketed preparation), and *C,* a control insulin preparation to which had been added a similar amount of zinc as in *A* and *B.*

Each preparation was tested on 20 rabbits. The concentration was 40 units per 1 cc. and each animal received 2 units of each preparation. The results of this experiment are summarized in figure 2.

3. The effect of aging on the clear solution of protamine zinc insulin preparation with reference to its hypoglycemic action was investigated on only one preparation. The tests were made when the preparation was 2, 3 and 4 days old. Each test was on 10 rabbits only. The switch-over technique was not employed. Each rabbit received 2 units of 40 units per
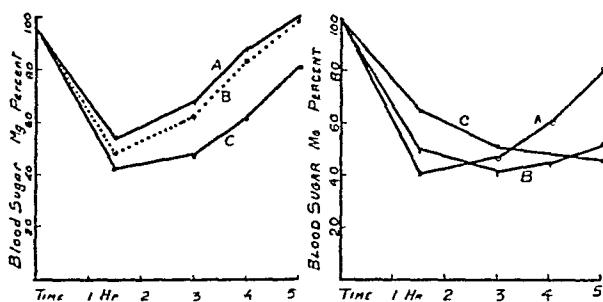
Fig. 1   Fig. 2

Fig. 1. The blood sugar of fasting rabbits following the subcutaneous administration of 0.5 unit per kilogram bodyweight of: *A,* 5 units per 1 cc. dilution of crystalline insulin; *B,* 5 units per 1 cc. dilution of crystalline insulin containing 0.2 mgm. zinc per 100 units, and *C,* 40 units per 1 cc. dilution containing 0.2 mgm. zinc per 100 units of crystalline insulin. Each curve represents averages of 20 rabbits.

Fig. 2. The blood sugar of fasting rabbits following the subcutaneous injections of 2 units of 40 units per 1 cc. dilution of: *A,* solution of crystalline insulin; *B,* clear solution of protamine zinc insulin and *C,* protamine zinc insulin pH 7.2 (marketed product). Each curve represents averages of 20 rabbits.

1 cc. and blood samples were removed at 0, 1.5, 3, 5, 7 and 9 hours respectively. The averages of the data are shown in figure 3.

4. The effect of sodium acid phosphate was investigated in order to determine whether or not its addition had any influence on the hypoglycemic action of the clear solution of protamine zinc insulin. Two preparations were made with and without buffer, and each preparation was tested on 26 rabbits using the switch-over technique. A summary of the results is found in figure 4.

DISCUSSION. It is sometimes difficult to demonstrate in the laboratory animal the prolongation of hypoglycemia following the parenteral administration of small doses of an insulin preparation endowed with prolonged activity. An example is the addition of 2 mgm. of zinc per 1000

units of insulin.   Scott and Fisher (4) showed that for all intents and purposes the addition of such an amount of zinc to insulin does not appreciably enhance its action on rabbits, and in an earlier publication from this laboratory (1) their findings were confirmed.   However, upon further investigation of this problem it was observed that the weak dilutions of this particular amount of insulin and zinc were responsible for the masking of its true action.   It was also shown (1) that the addition of 2 mgm. of zinc per 1000 units of insulin in solution with the adjustment of the hydrogen ion concentration to about pH 7.0 caused the precipitation of about 75 per cent of the insulin protein, or preferably stated about neutrality 75 per cent of the insulin was adsorbed on the basic zinc salt.   In view of this finding such an amount of zinc should appreciably prolong the action
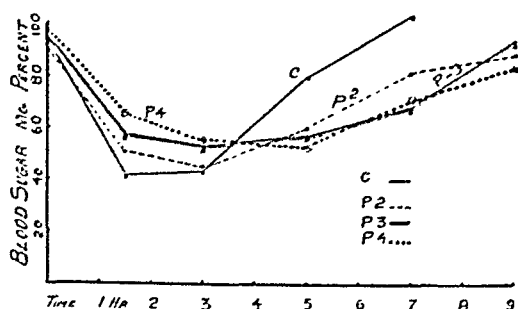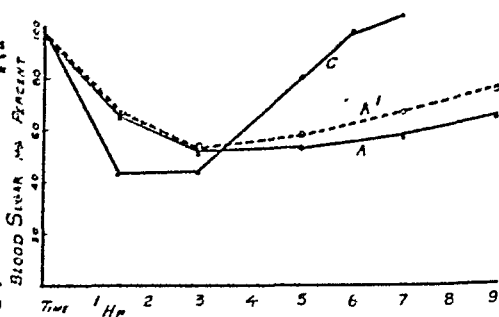
Fig. 3   Fig. 4

Fig. 3. The blood sugar of fasting rabbits following the subcutaneous injection of 2 units of 40 units per 1 cc. dilution of clear solution of protamine zinc insulin. P-2—two days old, P-3—three days old and P-4—four days old preparations. Each curve represents the averages of 10 rabbits.

Fig. 4. The blood sugar of fasting rabbits following the subcutaneous injections of 2 units of 40 units per 1 cc. of buffered, A, and non-buffered, A', clear solution of protamine zinc insulin. C, control crystalline insulin with zinc. Each curve represents the averages of 26 rabbits.

of insulin hypoglycemia in rabbits and some means should be discovered to make it demonstrable.   Several experiments were performed that were of doubtful significance, until the concentration of the material to be injected was studied.   Thus it became evident as shown in figure 1 that the administration of 2 units of insulin with zinc (2 mgm. per 1000 units) in concentration of 40 units per 1 cc. caused greater prolongation of hypoglycemia in rabbits than 2 units of the same preparation similarly given in concentration of 5 units per 1 cc.

Applying the well known physiologic principle of Donnan's theory of equilibrium to acidified solutions of insulin subcutaneously injected, neutralization at the site of injection must occur before absorption takes place. Basically, the principle involved in all preparations of insulin endowed with

prolongation of action is based on the decrease of their respective solubility at about the pH of the tissue fluid. The more insoluble a preparation is the greater its prolongation of action. Thus it was considered that an acidified mixture of protamine zinc insulin in a clear solution would cause a prolongation of hypoglycemia if the proper proportions of protamine to insulin were established. This was investigated in this laboratory and it was found that about 5 mgm. of protamine per 100 units of insulin with added zinc (0.2 mgm. per 100 units) yielded good results. The additions of 2.5 mgm., 10 mgm. and 20 mgm. of protamine to insulin were also studied, with somewhat similar results as shown in this paper. The various curves presented are self explanatory. *In vitro*, the amount of protamine necessary to cause the complete precipitation of the insulin protein is in the neighborhood of 0.75 mgm. to 1.25 mgm. per 100 units at about neutrality (4). *In vivo*, during the process of adjustment of ions of the clear solution of protamine zinc insulin at the site of injection the ratio of protamine to insulin should be greater since the size of the protamine molecule is considerably smaller than that of the insulin and undoubtedly a portion of the protamine not only would diffuse faster but there would be the possibility that it would combine with some of the tissue proteins. Consequently when equilibrium is reached it is necessary to have an adequate supply of free protamine to form the protamine zinc complex in the insoluble state at the site of injection.

Aside from the prolongation of hypoglycemia of the clear solution of protamine zinc insulin the data presented in figure 2 show the rapidity of the onset of its action as compared with the pre-precipitated protamine zinc insulin. This is clinically desirable as it would no longer be necessary to administer to the diabetic patient an adjunct dose of a fast acting insulin to take care of the blood sugar for the first few hours following the injection of protamine zinc insulin (pH 7.2).

The addition of phosphate to insulin preparations discussed in this paper seemed to enhance their action as shown in figure 4. However, the difference was not clear cut.

Unpublished data on the stability of the clear protamine zinc insulin in question are indeed favorable. Samples were incubated at 52°C. for 9 days with little loss in potency. Obviously this is very desirable.

SUMMARY

A method is described for the preparation of a clear acidified solution of protamine zinc insulin containing 5 mgm. of protamine and 0.2 mgm. zinc per 100 units. Its subcutaneous injection into fasting rabbits causes a prolonged hypoglycemia greater than that observed following the injection of a similar amount of crystalline insulin with and without added zinc.

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THE ACTION OF ALTERNATING CURRENTS UPON THE SPIKE-POTENTIAL MAGNITUDE, CONDUCTION VELOCITY AND POLARIZATION OF NERVE

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In previous communications from this laboratory (Rosenblueth and Reboul, 1939; Reboul and Rosenblueth, 1939) some effects of alternating currents (a.c.) upon nerve were reported. The present study deals with the changes produced by a.c. in the magnitude of the spike potential, the rate of conduction and the polarization of mammalian A fibers.

METHOD. The animals used were cats. They were anesthetized with dial (Ciba, 0.75 cc. per kgm.). The nerves studied were mainly the peroneal, occasionally the popliteal or the saphenous. They were dissected over as long an unbranched stretch as possible and placed in a moist chamber.

For studies of spike-potential magnitude or of conduction velocity the electrodes, both stimulating and recording, were silver or silver-silver chlorided wires. In the observations on changes of polarization the electrodes were calomel half-cells connected to the nerve through an agar-Ringer bridge.

Stimulation by single shocks was made with condenser discharges of various capacities and intensities. Repetitive stimulation was effected by condenser discharges through a thyratron, the frequency being controlled by a.c. These discharges were passed through a transformer before delivery to the nerve, in order to render them diphasic.

The sources of a.c. were two beat-frequency oscillators, a General Radio Company type 713B and a Clough Brengle Company model 79D. When the information was necessary a voltmeter indicated the output E.M.F., and a Weston thermocouple milliammeter, model 425, showed the intensity of current flowing through the nerve.

The spike potentials were led to a 5-stage capacity coupled amplifier and recorded from a cathode-ray oscillograph. When the recording electrodes were close to the region of the nerve where a.c. was applied, a capacity (0.01 to 0.1 μ F) was placed in parallel with the leads to the amplifier in order to reduce the a.c. artifact and the blocking effect of a.c. upon the amplifier. Although this procedure distorted the records, differences in magnitude or in delay were readily appreciated.

The changes of polarization were sometimes recorded on the cathode-ray oscillograph, after 3 to 5 stages of d.c. amplification. Tests of the electrodes and of possible thermal effects of large overloading a.c. voltages on the amplifier were made by substituting for the living nerve either the same nerve after killing it by immersion in hot water or a wick soaked in Ringer and repeating the experimental procedures used on the living nerve. A d'Arsonval galvanometer was also frequently used to record changes in polarization. Here again, tests made with dead nerves or with the living tissue, but after crushing between the a.c. and the lead-off electrodes, separated the genuine changes of polarization from possible spurious effects.

RESULTS. *Spike-potential magnitude.* Several precautions are necessary for the analysis of the changes of amplitude of the spike potential of a nerve trunk produced by applications of a.c. Thus, a decrease of the recorded spike-potential would result if some of the fibers failed to become active at the region of the recording electrodes, even when the spike potential of the active fibers could be full-sized or even increased in magnitude. Since a.c. may block the nerve impulses (Wedensky, 1903), a reduction in the number of active elements could in certain conditions be due to this block. A.c. may decrease the electrical excitability of nerve (Wedensky, 1903); test stimuli might therefore activate fewer elements during than before the passage of the current. Finally, as will be shown below, a.c. may decrease the conduction velocity of nerve impulses; an apparent reduction of the spike-potential magnitude could hence be merely a manifestation of temporal dispersion of the impulses corresponding to the different fibers in the nerve.

Two procedures were used to circumvent these sources of error. The first was to compare the magnitudes of the spike potential at various points beyond (with respect to the direction of the nerve impulses) the region where a.c. was applied. For convenience in describing the location of the several electrodes and their mutual distances a schema of the moist chamber is pictured in figure 1. If maximal test stimuli were delivered from a_1 to b_1 and a.c. was applied, for example, from c_1 to d_1, the responses recorded from any of the electrodes d_2, e_1 . . . . h_1 to the crushed region of the nerve at h_2 could only be due to fibers which had not been blocked by the a.c. Temporal dispersion would tend to decrease progressively the magnitude of such responses recorded in that order. If, then, the response at e_1 should be smaller than that at h_1 during the passage of a.c., it may be safely inferred that a.c. produces a decline of spike magnitude per fiber. This was readily found to be the case.

In figure 5 are illustrated results from a typical experiment. The lower records show the responses of the nerve at points 0.5, 1.5 and 3.5 cm., respectively, from one of the a.c. poles, during the passage of 15,000 cycles,

0.2 mam. The upper records show the control responses at the same points but without any a.c. It is apparent that the spike potential was more decreased in the neighborhood of the site of application of a.c. than in more distant regions of the nerve.

Figure 2 illustrates typical changes of spike magnitude at several points of a nerve, interpolar, polar and extrapolar, during the passage of a.c. of various intensities. It is clear that the depression is maximal at the a.c. poles, and becomes progressively less for more distant points. The magnitude of the response in the interpolar segment was always greater than at the poles; indeed, as shown in the figure, it was greater than at points at the same distance from one of the a.c. poles, but on the extrapolar region of the nerve. This apparent discrepancy is readily explained by the assumption that the passage of a.c. has not only a depressant effect upon the spike magnitude, but that it also has an independent increasing action, more visible in the interpolar segment than in neighboring extrapolar regions.

Although a significant increase of spike magnitude above the control level was never seen, the recognition that a.c. may cause such an increase explains both the changes in the interpolar segment and the complex shape of the curves in figure 2 for the extrapolar regions.

The second method employed to study changes in spike magnitude was as follows. The nerve was stimulated at one end and the recording electrodes were placed on intact regions in the middle of the excised trunk, so that a diphasic response was obtained. Applications of a.c. on either side of the recording electrodes should then result, if a.c. has a depressing effect more marked near the poles than in more distant regions, in a preferential decrease of one or the other of the two components of the diphasic record. This was found to take place (fig. 3).

With either of the two methods used for determining changes of spike-potential magnitude, the degree of the decrease varied proportionally with the intensity of a.c. applied. An influence of frequency was likewise recognizable. When different frequencies were applied with constant intensity lower frequencies (e.g., 200 to 2,000 cycles per sec.) had a greater depressing action than did higher frequencies (e.g., 2,000 to 15,000 per sec.).

Changes in conduction velocity. Certain difficulties have again to be considered for the interpretation of records showing an apparent slowing of the nerve responses during or after applications of a.c. Thus, if the fastest fibers in the nerve were more readily blocked than elements of slower conduction rate, a comparison of the measurements of conduction velocity taken before and during the passage of the current might not be legitimate, for such measurements could apply to different elements in the nerve.

That a.c. can slow considerably the conduction rate is clear, however,

from the following data. The responses illustrated in figures 3, 4 and 7 show not only a more delayed beginning during or after than before a.c., but also a delayed subsidence. The latter delay may be safely interpreted
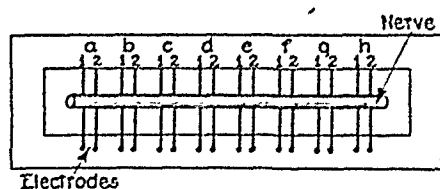
Fig. 1
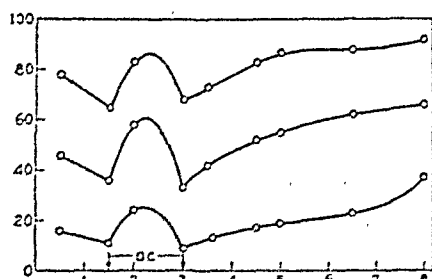
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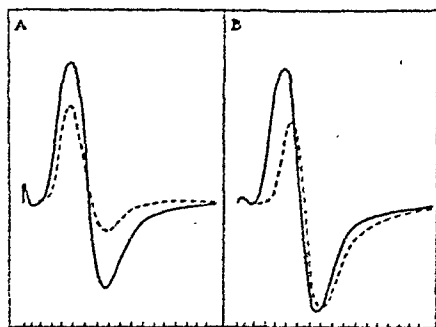
Fig. 3
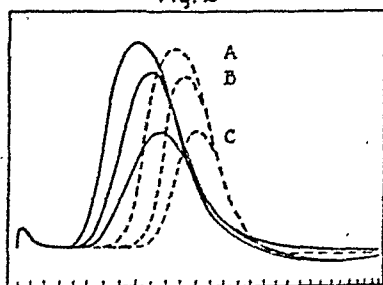
Fig. 4

Fig. 1. Schema of the moist chamber. The electrodes are arranged in pairs. The distance between the members of each pair (i.e., from a_1 to a_2; from b_1 to b_2; etc.) is 0.5 cm. The distance between neighboring pairs (i.e., from a_2 to b_1; from b_2 to c_1; etc.) is 1 cm.

Fig. 2. Changes of spike magnitude during the passage of a.c. (frequency 15,000 cycles per sec.). Ordinates: spike magnitude during the application of a.c. expressed as per cent of the responses obtained at the corresponding points before a.c. Abscissae: centimeters. The stimulating cathode was at O; the arrows indicate the points in the nerve at which the a.c. was delivered. Average voltage of a.c.: A, 1 v.; B, 2.2 v.; C, 3.2 v.

Fig. 3. Changes of spike magnitude during the passage of a.c. (frequency 15,000 cycles per sec.). Stimulating electrodes at a_1 and b_1 (see fig. 1). Diphasic records from d_2 to e_2. The records were drawn by projecting the original film through a photographic enlarger. Solid lines: control responses without any a.c. Broken lines: responses during delivery of a.c. A, a.c. (2 v.) applied from e_2 to f_2. B, a.c. (1.5 v.) applied from c_2 to d_2. Time scale: 0.2 msec.

Fig. 4. Changes of spike magnitude and of conduction velocity during the passage of a.c. of progressively increasing intensity (A, B, C, solid lines) and progressively decreasing intensity (C, B, A, broken lines). The records were drawn by projecting the original film through a photographic enlarger. Time scale: 0.2 msec.

as indicating a slowing of some of the originally slow elements in the nerve, dismissing as unlikely the alternative possibility that the time course of the spike potential per fiber should be greatly modified by a.c.

Measurements of the conduction rate for different segments (e.g.,

1.5 to 3.0 cm.) of the interpolar and distal extrapolar region of the nerve clearly indicate, likewise, that a marked slowing may take place. The conduction rate of the fastest elements responding beyond the site of application of a.c. increases progressively with the distance from the a.c. electrodes. Thus in a typical experiment the stimulating electrodes were placed at a_1 and b_1, and the a.c. electrodes at c_2 and c_1. The conduction velocity measured for different segments of 2.5 cm. along the nerve was uniformly 70 m. per sec. before a.c. was applied. During the application of 15,000 cycles per sec. (5 v.) the conduction velocities measured for the
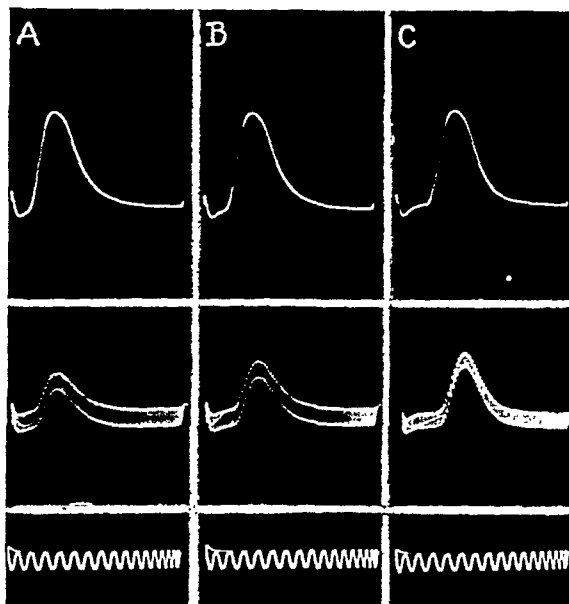
Fig. 5. Changes of spike magnitude during the passage of a.c. Stimulating electrodes at a_1 and b_2. A.c. electrodes d_1 and e_1. Records: A from e_2, B from f_1 and C from g_2, to the crushed end at h_2. Upper records: controls without a.c. Lower records: during the passage of a.c. (frequency 15,000, 1.2 v.). Time signal: 2,000 cycles.

segments from c_2 to e_1 (interpolar segment), from d_2 to f_1, and from e_2 to g_1 were approximately 45, 50 and 60 m. per sec., respectively.

As was the case for changes of spike magnitude, the degree of slowing produced by a.c. was greater for more intense than for weaker currents. With constant intensity relatively low frequencies were more effective than higher frequencies.

Changes of polarization. Applications of a.c. may change markedly the resting polarization of nerves. The changes at or near the a.c. poles are detectable with reference to more distal regions of the nerve or as changes of the demarcation potential. The results were complex. Relative positivity or relative negativity of the region treated by a.c. or of neighboring segments could be recorded.

In general, the sign and magnitude of the polarization change depended on the intensity and frequency of the a.c. applied and on the distance of the region of the nerve investigated from the site of application of a.c., as follows. When, with constant frequency, the intensity of the a.c. applied was progressively increased, the regions of the nerve at the poles or in the close neighborhood first became progressively more negative with respect to a distant crushed point (cf. Bishop, 1932). Further intensification resulted, however, in decreased but more prolonged negativity,
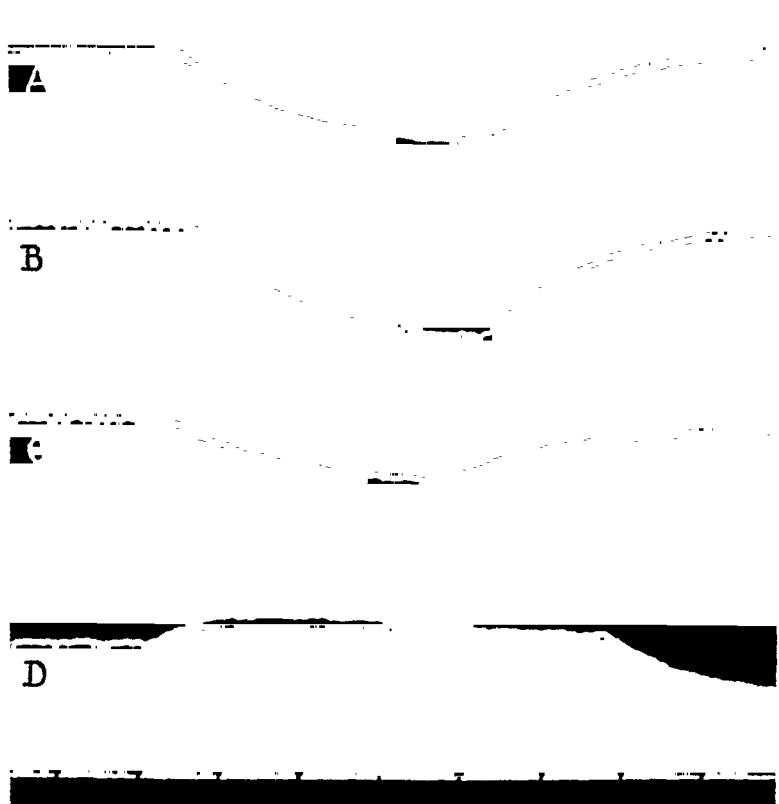
Fig. 6. Changes of polarization. A.c. electrodes at a_1 and c_1. Leads to the galvanometer from c_2 and e_1. Downward excursions denote negativity of c_2 with respect to e_1. The a.c. applied was in all cases with a frequency of 200 cycles per sec. The voltages were A, 1; B, 3; C, 7; and D, 17. Time scale: 1 sec. Crushing the nerve between c_1 and c_2 resulted in the disappearance of all the changes recorded.

as compared to the effects of weaker currents (fig. 6). Stronger intensities led usually to positivity during the passage of the current, followed by prolonged negativity after cessation of the a.c. (fig. 6D, see Wedensky, 1903).

The influence of the frequency of the a.c. was readily seen by the application of different frequencies with a constant intensity. The effects with relatively high frequencies (e.g., 15,000 cycles per sec.) were much smaller than with slow frequencies (e.g., 200 per sec.). If, however, the

high frequencies were made sufficiently intense the results were similar to those obtained with weaker, slower frequencies.

The distance from the a.c. poles was likewise an important factor in determining the sign and magnitude of the changes of polarization observed. Thus, when a strong current was applied, so that the region of the nerve at the poles or in the close vicinity became relatively electropositive, regions slightly more distant from the poles could register electronegativity with respect to a far, crushed reference point.

The independence of the several effects of a.c. upon nerve. It was interesting to attempt to correlate the effects of a.c. upon the different aspects of nerve function studied here with each other and with the effects of electrical excitability described previously (Reboul and Rosenblueth, 1939). It was also interesting to investigate whether or not the effects of a.c. could be correlated with the well-known electrotonic actions of d.c. A specific question which arises in analyzing such a possible correlation is the following. Is decreased polarization always associated with decreased spike magnitude and increased electrical excitability and conduction velocity, as the data on electrotonus and the membrane theory suggest? Several experiments were planned to answer this question.

That the changes of spike magnitude may be independent of the changes of conduction velocity is indicated by the following observations. In figure 4 are illustrated typical effects upon the nerve responses when a.c. was applied continuously for a prolonged period, first with progressively increasing and then with progressively decreasing intensity. The stimulating electrodes were placed at a_1 and b_1 (fig. 1); a.c. was delivered from d_1 to f_1 and the record was taken from g_1 to h_1. The solid lines show the responses during the application of progressively more intense a.c., and the dotted lines the responses when the a.c. was gradually weakened. Although the record was not taken at one of the a.c. poles, where the slowing was probably maximal, the discrepancy between the effects on spike magnitude and on conduction velocity, respectively, in the responses during progressive intensification as compared with the responses during gradual weakening of the a.c., clearly indicates the independence of the two depressive actions.

A similar discrepancy between the recovery of spike magnitude and that of conduction velocity is illustrated by a different method in figure 7. The stimulating electrodes were at a_1 and b_1; the a.c. electrodes from e_1 to g_1; and the records were taken from g_1 to h_1, that is, from the region at one of the a.c. poles to the crushed distal end. The large rapid response is the control before application of a.c. The progressively increasing slower spikes were photographed at about 3-sec. intervals after a.c. (5,000 cycles per sec.; 0.6 mam.) had been applied for 5 sec.—i.e., during recovery from complete block.

The facts which indicate that spike magnitude and conduction velocity may vary independently may be summarized by the statements that as a rule weak currents can have striking action on spike magnitude with only slight effects upon conduction velocity, and after prolonged or strong applications of a.c., the slowing of conduction can long outlast the depression of spike magnitude.

That the electrical excitability of nerve can vary independently of the changes in spike magnitude and in conduction velocity is shown by the following observations. Reboul and Rosenblueth (1939) found that the electrical excitability of the extrapolar regions of the nerve near the a.c.
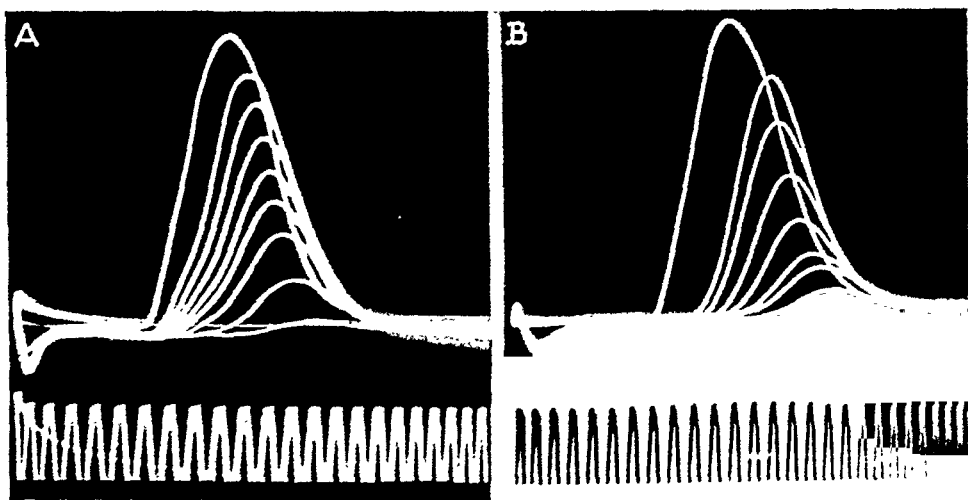
Fig. 7. Independence of the recovery of spike magnitude and of conduction velocity after a strong application of a.c. Stimulating electrodes at a_1 and b_1. A.c. electrodes at c_2 and g_2 for A, and at d_2 and f_2 for B. Records from g_2 and f_2 for A and B, respectively—i.e., from the region of the a.c. pole—, to the crushed region at h_2. The responses were photographed without moving the camera. The largest and quickest control response was first taken. A.c. (frequency 5,000 cycles per sec., intensity 0.6 mam.) was then applied for 5 sec. Further pictures were then taken at about 3-sec. intervals as the responses, which had been entirely cancelled by the a.c., progressively grew back toward normal. Time calibrations: 5,000 cycles.

electrodes decreased when weak a.c. was applied and increased upon application of strong a.c. The present study, on the other hand, shows that the spike magnitude at those regions decreases progressively as the a.c. is intensified. This absence of correlation was tested on a given nerve as follows. Stimulating electrodes were placed at a_1 and b_1 and a.c. electrodes at c_1 and d_1. The maximal spike magnitude was recorded at e_1, by leading from that point to the crushed region at h_2, before and during the passage of a.c. with a fixed frequency and various intensities. The changes of electrical excitability at e_1 were then measured by sending submaximal condenser discharges of constant capacity and amplitude

from d_2 to e_1 (cathode) and recording the responses from g_2 to h_2 before
and during the passage of a.c. with the same frequency and intensities
that had been used before.   The results were as usual; the spike magnitude
progressively decreased as the a.c. was intensified, while the electrical
excitability first decreased and then increased at the point tested.

The data on changes of polarization (fig. 6) clearly indicate the inde-
pendence of these changes from those of spike magnitude and conduction
velocity.   As a.c. of a given frequency is progressively intensified spike
magnitude and conduction velocity gradually decline, while relative
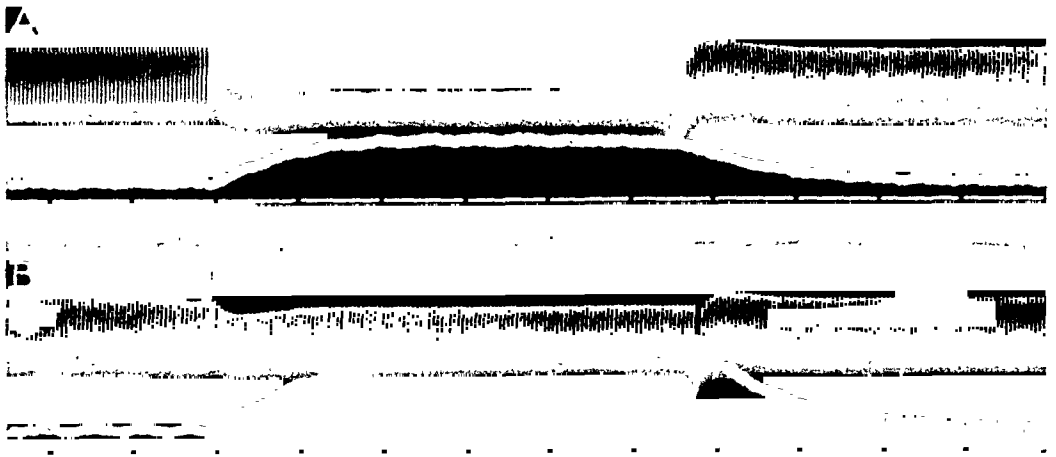
Fig. 8. Changes in spike magnitude and in polarization produced by a.c. and by
d.c. (catelectrotonus).   Stimulating electrodes at a_1 and b_1.   A.c. or d.c. electrodes
(impolarizable) at d_1 and e_1.   Recording electrodes (impolarizable) at e_2 and f_2.
The leads from these electrodes were made in parallel to the galvanometer and the
capacity-coupled amplifier.   Two additional capacities (0.1 μ F) were placed in each
of the leads to the amplifier to prevent any d.c. interference from this source to the
galvanometer.   Upper tracing: nerve spike-potentials recorded from the cathode-
ray oscillograph.   Lower tracing: galvanometer; upward excursions denote relative
negativity of d_1 with respect to e_1.   Time: 1 sec.
A. A.c., frequency 10,000, average amplitude 9 v.
B. D.c., 0.5 v.

negativity first increases and later decreases to be substituted in some
cases by relative positivity.   This independence is also emphasized by
the comparison of the effects of a.c. with those of d.c.   In a series of ob-
servations, with stimulating electrodes at a_1 and b_1, a.c. or d.c. was applied
from c_2 to e_2 and the responses were recorded monophasically from f_1
to h_1.   Typical results are illustrated in figure 8.   Invariably when the
a.c. and the d.c. were adjusted so that approximately equal changes of
polarization ensued the spike magnitude was more and longer depressed
by a.c. than by d.c.

A comparison of changes of polarization with changes of electrical excitability again reveals absence of correlation. Throughout the range of intensities of a.c. which result first in a decrease and then in an increase of electrical excitability the region of the nerve tested may show relative negativity with respect to a distant reference point.

DISCUSSION. There is no reason to assume that a.c. will have any effects on nerve qualitatively different from those produced by d.c. It would be expected, therefore, that the action of a.c. should be explainable as an interaction of anelectrotonus and catelectrotonus.

Prolonged applications of a.c. have more marked effects than brief applications. It is obvious, therefore, that the anelectrotonic and catelectrotonic effects corresponding to each half-cycle, respectively, do not cancel their effects. The conclusion is supported by the fact that the aftereffects of a.c. of a given frequency and intensity are consistent—i.e., they do not depend on the sign and amplitude of the current at the time when it is stopped.

If anelectrotonus and catelectrotonus correspond to actions of the current of opposite sign on the same (one or several) structures or processes in nerve a lack of cancellation would take place if the cathodal and anodal effects are asymmetric in their amplitude, their spatial distribution or their time course. Such asymmetry was established by Pflüger (1859) and has been abundantly confirmed. With weak d.c. the cathodal effects are more prominent and widespread than the anodal effects. Strong currents lead to the opposite result. It would be expected accordingly that weak a.c. might be similar in action to catelectrotonus, while strong a.c. would duplicate the results of anodal polarization. In table 1 are summarized some effects of weak or strong a.c. or d.c. It is apparent that the expectation mentioned is not confirmed.

Two alternative views are compatible with the data. The cathodal and anodal effects may not be due to opposite actions on the same processes or structures but might be caused by influences exerted at different steps in the nerve. In such a case they could co-exist independently without mutual cancellation. The second suggestion is that the cathodal and anodal actions are not simple but complex. Thus, during weak catelectrotonus there might be not only an influence increasing the electrical excitability but also a marked tendency to decrease this excitability. This view could account for the reversal of effects which commonly takes place upon cessation of the d.c. applied, a reversal which has not received thus far an adequate explanation. There is no evidence available to support or reject either of these two hypotheses.

The discussion has dealt thus far with the effects of the electric current upon nerve. It is now interesting to consider the bearing of the data on the problem of the interrelation of different aspects of nerve function.

The data illustrated in figures 4, 7 and 8, and those summarized in table 1 emphasize the large degree of independence of the several features of nerve function studied. Conduction velocity depends probably on at least two factors, the amplitude of the stimulus delivered by the active region and the excitability of the succeeding segments. It is not difficult, therefore, to conceive that it might vary independently of the changes in either of these two factors considered singly.

The membrane theory suggests, on the other hand, that the spike amplitude should vary directly, and the electrical excitability inversely as the demarcation potential. The present data do not support this suggestion. Other facts can be quoted in support of the independence of the variables in question. Thus, as shown by Bishop and Erlanger (1926), a rise of temperature increases the demarcation potential and may increase the spike magnitude, but it results in an increase, instead of a decrease, of electrical excitability. Similarly, Bishop (1932) found that cocaine, which

TABLE 1

A comparison of some actions of a.c. with the corresponding effects of d.c.

The signs indicate: +, increase; 0, no change; −, decrease

	DEMARCATION POTENTIAL	SPIKE MAGNITUDE	ELECTRICAL EXCITABILITY	CONDUCTION VELOCITY
Weak a.c.	−	−	−	−
Strong a.c.	0, +	− −	+	− −
Weak catelectrotonus	−	−	+	+
Strong catelectrotonus	− −	− −	−	−
Weak anelectrotonus	+	+	−	−

produces no significant change in the amplitude of the spike potential, increases slightly the demarcation potential and reduces markedly the electrical excitability.

A theory of nerve conduction that covers the data is not available. For the present the suggestion which emerges is that if the three variables discussed are functionally related, as there is good reason to believe, the expressions for this relationship should include at least two independently variable parameters, to allow for the number of degrees of freedom in the system. Thus, if the development of the spike potential corresponds not to a total depolarization of an interphase but to a partial breakdown, capable of independent variation, the observed independence between the demarcation potential and the amplitude of the spike could be readily accounted for. Similarly, if the electrical excitability should be interpreted as depending not exclusively on the dgree of initial polarization but also on some additional independent factor, such as the rate of movement of

the ions concerned, then the independent variations of the demarcation potential and the electrical excitability would be explained.

SUMMARY

Applications of alternating currents (a.c.) to nerves result in a decrease of the amplitude of the spike potential at and in the neighborhood of the a.c. poles (figs. 2, 3 and 5).

The conduction velocity of nerve is slowed by a.c. (figs. 3, 4 and 7).

The demarcation potential is either decreased or, less commonly, increased by a.c. (figs. 6 and 8).

These several changes and those of electrical excitability (Reboul and Rosenblueth, 1939) may occur independently (figs. 4, 7 and 8; table 1).

The data are discussed from the standpoint of the problem of the actions of electric currents on nerves and from that of the problem of the functional relationship between the demarcation potential, the spike-potential amplitude and the electrical excitability of nerve.

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DECREASE OF HISTAMINASE IN TISSUE BY ADRENALECTOMY AND ITS RESTORATION BY CORTICO-ADRENAL EXTRACT

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In a previous communication a method for the determination of histaminase in the tissues of the rat was given (Rose, Karady and Browne, 1940). It was shown that the only tissues containing the enzyme in this species are lung and intestine. It is well known that the resistance of the rat and other species to histamine is markedly diminished following adrenalectomy (Gottesman and Gottesman, 1928) (Rose and Browne, 1938). It has further been shown that the administration of cortin to an adrenalectomized rat will restore the resistance of this animal to histamine to normal (Gottesman and Perla, 1931) and the ability to inactivate histamine (Rose, 1939).

In the following experiments, the effect of adrenalectomy on the histaminase content of rat lung was studied. A preliminary report of this has already been given (Rose and Karady, 1939). Further observations on the effect of treatment with cortin on the histaminase content of the lung of the adrenalectomized animal are reported in the present communication.

METHODS. Rats both male and female of a hooded strain weighing from 140 to 180 grams were used. Under ether anesthesia, bilateral adrenalectomy was performed and after allowing the animals to recover, they were placed on a purina diet and given normal saline to drink. After an interval of 7 to 10 days, the time at which the diminished resistance to histamine is known to be well established (Rose and Browne, 1938) the animals were again anesthetized with ether, and observing sterile precautions, the lungs were removed, washed free of blood in normal saline, and then placed on large sheets of filter paper to remove the excess moisture. At the same time, lung tissue from intact animals was removed in a similar manner. The lungs of all the animals in each group were then ground up, mixed, and an accurately weighed amount, usually 0.5 gram, placed in Erlenmeyer flasks of 75 cc. capacity containing 20 cc. of phosphate buffer of pH 7.2. To this was added 1 cc. of a histamine HCl solution

containing 1000 γ of histamine base per cubic centimeter. After the addition of 10 drops of Toluol, the flasks were placed in an incubator at 38°C. for 16 hours. They were then removed, heated to 90°C. for five minutes and then made up to 50 cc. final volume. The histamine content was determined by the guinea-pig-ileum method.

RESULTS.   a. *Effect of adrenalectomy.* Five groups of 5 animals were used in this series. The first three were maintained on saline for seven days, and then used. The results are shown in table 1. The figures

TABLE 1

The effect of adrenalectomy on the ability of rat lung tissue to inactivate histamine

EXPERIMENT NUMBER	CONTROLS		ADRENALECTOMIZED	
	Weight of tissue	Amount histamine destroyed	Weight of tissue	Amount histamine destroyed
			Maintained on normal saline for 7 days	
	grams	γ	grams	γ
157	0.5	800	0.5	600
	0.5	780	0.5	600
			0.5	600
158	0.4	750	0.6	750
	0.4	750	0.4	650
	0.2	400	0.2	300
159	0.5	800	0.6	700
	0.5	750	0.5	700
			0.2	300
			Maintained on saline for 7 days and on water for 3 additional days	
164	0.5	800	0.5	400
	0.5	700	0.5	400
			0.5	300
167	0.5	800	0.5	400
	0.5	700	0.5	400
			0.5	300

Tissue incubated with 1000γ histamine for 16 hours.

given denote the amount of histamine destroyed in 16 hours by varying weights of tissue. It will be observed that in experiments 157, 158, 159 only a moderate decrease in the histaminase content occurred (approximately 20 per cent). Since it is known that adrenalectomized rats may be maintained for long periods of time on normal saline alone, it was decided to place the animals on water for three additional days. Such animals become quite weak and die within 4 to 5 days. The results of this procedure on the histaminase content of lung are shown in experiments 164 and 167,

table 1, and experiments 170, 182 and 188, table 2. It will be noted that there is a marked diminution in the histaminase content of the tissue, 40 per cent of the histamine being destroyed as compared to 75 per cent in the same time by an equal weight of tissue from intact animals.

b. *Effect of administration of cortin.* Six groups of adrenalectomized animals were used. They were maintained on normal saline for seven days. Three of these were then given 2 cc. of Wilson's Cortin[2] intramuscularly twice daily for 4 days and the saline replaced by water. On the 12th day the animals were killed and the tissues removed in the usual manner. The other three groups were taken off saline on the 7th day and

TABLE 2

The effect of the administration of cortin to adrenalectomized rats on the ability of lung tissue to inactivate histamine

EXPERIMENT NUMBER	HISTAMINE DESTROYED BY 0.5 GRAM OF LUNG TISSUE		
	Controls	Adrenalectomized, maintained on normal saline 7 days and on water for 4 days	Adrenalectomized, maintained on normal saline 7 days and on cortin for 4 subsequent days plus water to drink
	γ	γ	γ
170	750	400	600
		450	500
		300	700
			750
			700
182	700	400	600
		500	600
		300	600
			600
188	650	500	700
		525	700
		500	700
			700

Tissue incubated with 1000γ histamine for 16 hours.

placed on water for the remaining four days, at which time they were used. There were also three groups of intact animals whose lung tissue was removed and incubated in the same manner. The results are shown in table 2.

It will be observed that the ability of the lung tissue of adrenalectomized animals to destroy histamine is restored by the administration of cortin in the amounts indicated, so that an average of 65 per cent of the added histamine is inactivated by 0.5 gram tissue.

[2] Supplied through the courtesy of Dr. D. Klein and the Wilson Laboratories, Chicago.

DISCUSSION. The decrease in resistance to histamine following adrenalectomy may be explained in part by a decrease in the histaminase content of tissue, based upon the above results. Since in the rat, the major part of histamine inactivation takes place by the kidney and liver (Rose and Browne, 1938), and since neither of these two organs contains histaminase in this species (Rose, Karady and Browne, 1940) it does not seem likely that the decrease in histaminase is a major factor in causing the delay in the destruction of injected histamine in the adrenalectomized animal. It may, however, play a minor rôle. Furthermore, the adrenalectomized rat maintained on normal saline is markedly sensitive to histamine as compared to the normal intact animal and, as noted above, there is only a moderate diminution in the histaminase content of the tissue, unless the adrenalectomized animals are deprived of normal saline. It is also interesting to note in this connection that there is an increase in the histamine content of the small intestine and stomach of adrenalectomized rats, and that this increase is even greater when the animals are given water instead of normal saline to drink (Rose, 1940).

It has been shown that the ability of the adrenalectomized rat to destroy histamine may be restored to normal by the administration of adequate amounts of cortin (Rose, 1939). This may also be in part accounted for by the above results. It is known that anaphylactic shock is much more readily produced in the adrenalectomized rat than in the intact animal (Wyman, 1929). This might be explained in part by the fact that there is a decrease in histaminase and an increase in the available histamine in certain tissues.

CONCLUSIONS

The effect of adrenalectomy on the histaminase content of the tissue of the rat has been studied. It has been shown that if animals are adrenalectomized, maintained on a standard diet, and given normal saline to drink, there occurs a diminution of the histaminase content of the lung. If water is substituted for the normal saline, an even greater diminution occurs. The diminution of histaminase in the lung tissue of the adrenalectomized rat may be restored to within normal limits by the administration of adequate amounts of cortin. The significance of these findings is discussed.

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OPACITIES OF THE LENS INDUCED BY ADRENALIN IN THE MOUSE

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Rapidly precipitated transitory opacities of the lens have been noted in laboratory animals incidental to the following conditions: slow asphyxia —rat (1); "alarm reaction"—rat, cat (2); toxic substances (especially adrenalin)—rat, mouse (3). Extensive studies on the more slowly developed cataracts of metabolic origin have been published (galactose feeding, naphthalene, deficiencies in the components of the vitamin B_2 complex—cf. 4) but the acute reversible type has in all probability been passed over by many observers as toxic cataract of little significance in the physiology and pathology of the human lens. Biozzi (1) studied it in some detail from the physiological and histological standpoint. Nordmann (6, 7) in a résumé of his researches on cataract discussed it at length. In this and in the more recent review (5) are mentioned Collevati's observations on the regular occurrence of opacities following the administration of adrenalin, which apparently still lack confirmation.

Recently the 1909 report of Schultz (3) on the comparative activity of various adrenalin preparations has come to my attention, in which he describes in detail this toxic effect of adrenalin for the mouse lens. Although the frequency of its occurrence was not explicitly stated, his observations on the effective range of adrenalin dosage, the speed of onset, the complete reversibility of the cataract, and the unpredictable variation in the reaction of the mouse were in close agreement with the observations reported below.

METHODS. Originally the limited data available suggested that the change in the lens might be associated with a combination of adrenalemia and shock. Therefore the effects of single toxic intraperitoneal injections of adrenalin alone (adrenalin chloride, 1:1000 sol., Parke Davis), of histamine alone (ergamine acid phosphate, Burroughs Wellcome), or of histamine followed by adrenalin, were observed as regards changes in the lens in male and female mice (average weight 20 grams).

RESULTS. *Adrenalin.* Within 3 to 10 minutes from the time of injection of from 0.3 to 0.7 mgm. per 100 grams of the drug, the following train

of symptoms occurred: quietly increasing prostration, dyspnea, lachryma-
tion, salivation, cyanosis, exophthalmos, and in severe states reduction
or absence of the light reflex.   Individual variation was marked in some
animals (cf. 3), the severity of the reaction with the smallest amount of
adrenalin in some being equal to that with the maximum dose in others.
The degree and frequency of the response tended to be increased after
moderate fasting (4–6 hrs.), definite rigor appearing in a few.   The total
mortality for non-fasted mice averaged but 11.8 per cent in this range of
dosages but suddenly increased to 70 to 100 per cent starting at 0.8 mgm.
per 100 grams with an occasional mouse surviving 1.0 mgm., while the
total mortality of fasted mice reached 19.9 per cent because of the increased
number of delayed deaths, i.e., incomplete recovery and relapse (shock-
like death).

Single or double transitory opacities developed within the first hour after
the injection, with increasing frequency (from 10 per cent to 57 per cent)
as the dosage of adrenalin increased from 0.3 mgm. to 0.7 mgm.   The
average time of onset was 30 minutes, although changes in the lens were
often visible under magnification in 15 minutes.   In a few, 60 minutes
elapsed before the development of an initial diffuse cloud which rapidly
progressed in intensity with marked precipitation of the suture pattern.
The depth of the opacity varied with the individual from an irregular small
area of clouding with a partially defined suture pattern, to a heavy uniform
cloud with a dense "Y" formation, or the solid white marble eye noted by
others.   The duration of the cataract was proportional to the density
attained, a light opacity reaching maximum and clearing within 30 minutes,
whereas an intense cataract required about one hour to reach maximum and
two to three hours for dispersion.   The first signs of clearing started with
a more or less central loss (breaking) of the uniform turbidity with a slower
fading of the arms of the "Y".   In vivo and in vitro the whitish substance
seemed to recede toward the equator of the lens.

No consistent correlation was apparent between the intensity of the
cataract and the degree of collapse, exophthalmos, cyanosis, etc., an oc-
casional positive case retaining an active light reflex.

Biopsies of eyes showing varying degrees of opacities confirmed the gross
observations that the reaction was limited strictly to the lens.   The cloud-
ing was due to the formation of a subcapsular semifluid milky substance
confined to the superficial cells of the anterior cortex.   According to
Biozzi (1) no histological changes are found in the epithelium or the capsule
other than an occasional vacuolization of the superficial cortical fibres.
Since examination of a typical specimen of an adrenalin-induced opacity
confirmed this, further histological studies were postponed.

Histamine.   As may be seen from the table, the mice reared in this
laboratory showed the usual high resistance to histamine, 3 mice surviving

180 mgm. per 100 grams. There were no visible alterations in the clarity of the lens following injection.

Histamine and adrenalin. The injection of adrenalin (0.5 and 1.0 mgm. per 100 grams) was given 10 to 20 minutes after histamine before the characteristic symptoms of shock had appeared (circulatory stasis, cyanosis, exophthalmos).

TABLE 1

DESCRIPTION	HISTAMINE PER 100 GRAMS	ADRENALIN PER 100 GRAMS	NUMBER USED	APPEARANCE OF OPACITIES			DISAPPEARANCE OF OPACITIES		MORTALITY, < 1 HOUR		MORTALITY, ± 12 HOURS		SURVIVAL	
				Number	Per cent	Time	Number	Time	Number	Per cent	Number	Per cent	Actual number	Possible number
	mgm.	mgm.				min-utes		min-utes						
Normal		0.3	11	1	9.9	15-20	1	45	0	0	0	0	11	0
Normal		0.5	84	36	42.7	15-20	20	60-150	8	9.5	2	2.3	53	21
Normal		0.75	7	4	57.0	38-56	4	60-196	1	14.2	0	0	6	0
Normal		1.0	5	1	20.0	20	0	.	4	80.0	1*	20.0	0	0
Fasted ca 6 hours		0.5	54	33	61.1	20-40	22	60-120	3	5.5	8	14.4	18	25
Histamine	50-180	0	15	0	0	0	0	0	0	0	0	0	15	0
Histamine 15 minutes before adrenalin	12.5	0.5	8	6	75.0	15-23	6	54-75	0	0	0	0	8	0
	25.0	0.5	11	2	18.1	15-30	2	60-150	0	0	0	0	11	0
	50.0	0.5	28	3	10.7	15-50	3	70-130	1	3.6	1	3.6	26	6
	25.0	1.0	16	15	93.7	20-60	3	40-200	0	0	1	6.3	12	3
	35.0	1.0	13	10	76.9	30-90	8	50-230	0	0	1	7.6	9	3
	50.0	1.0	20	8	40.0	15-52	7	98-212	0	0	5	25.0	20	0
Gynergen II, 0.2 mgm.		0.5	8	0	0	0	0	0	0	0	0	0	8	0
Phloridzin, 1 mgm. fasted		0.5	13	0	0	0	0	0	0	0	0	0	13	0

* Died 2 to 3 hours after adrenalin injection.

From the table it may be seen that the injection of adrenalin superimposed upon a mild degree of histamine intoxication modified the toxic effect of adrenalin in three ways:

1. When the proportion of the histamine to that of adrenalin was about 25:1 the incidence of cataract increased 30 to 40 per cent above the maximum caused by the same amount of adrenalin alone. The formation of the opacity was in all respects identical with that described above. There was some indication of increased duration and intensity, but slight differences of this nature in animals with considerable variation are difficult to determine.

2. When the amount of histamine was increased, so that the ratio of the dosages of the drugs definitely exceeded 25:1, the incidence progressively declined.

3. The acute and rapidly fatal systemic reaction usually following such doses of adrenalin did not develop, the mortality dropping and all but one death occurring several hours after the injection. This change resembles alterations in the reaction to adrenalin intoxication observed in rats (15).

Blood sugar. From the glycogenolytic character of the inciting agent and the time of appearance of the opacity, the hyperglycemic reaction seemed to be implicated as the initiating factor of diverse and extensive systemic effects ultimately modifying the stability of lens metabolism. Furthermore in a short series of mice previously treated with ergotamine (Gynergen II, Sandoz) which inhibits adrenalin hyperglycemia, and in fasted mice after a single injection of phloridzin, no opacities could be produced. Therefore an attempt to obtain some comparative figures on the blood sugar in cataractous and non-cataractous mice seemed desirable. Because of the circulatory stasis during adrenalin intoxication reliable peripheral blood samples are not obtainable. The mice were sacrificed for the Hagerdorn-Jensen (HJ) micro-determinations on heart blood specimens. However, some preliminary attempts were made to follow roughly the blood sugar changes using the more rapid Folin-Malmros (FM) micro-method.

In untreated, unanesthetized, fed or semi-fasted mice, tail blood is easily obtained; but because of the excitability and restlessness of the species rapid elevation of the blood glucose occurred frequently, ranging from 68 to 172 mgm. per cent HJ or from 112 to 234 FM. In apparently quiet mice, however, the average for 3 cases was 94 mgm. per cent HJ and for 5 cases 116 mgm. per cent FM.

Blood sugar determinations on heart blood of non-cataractous mice, 20 to 40 minutes after the injection of adrenalin (0.5 mgm.), showed more uniformity between individuals and more agreement between the two methods. In 5 cases the average was 220 mgm. per cent HJ (range—156 to 279), and in 4 cases the average was 225 mgm. per cent FM (range—172 to 292). In cataractous mice under the same conditions the blood sugar in 7 cases averaged 123 mgm. per cent HJ (range—87 to 181), but by the FM method the blood reducing values in 12 cases averaged 412 mgm. per cent (range—252 to 600). According to the Hagerdorn-Jensen determinations, therefore, there appeared to be an unexpected but consistent low "true" glucose level in the cataractous mice during the period when a marked hyperglycemia due to adrenalin should occur, while the high values obtained by the Folin-Malmros method indicated a spectacular rise in the non-glucose reducing fraction.

DISCUSSION. In the adrenalin-induced cataracts in mice the speed of onset, the reversibility, and the opaque, hydrated state of the anterior cortical tissue are grossly similar to the initial stages of cataracts associated with carbohydrate disturbances as described by others (galactose cataracts

in rats—9, 10, 11; naphthalene cataracts—4, 12; certain senile varieties in the diabetic human—9, 13; the rare cataracta diabetica—14). Physiologically they are elicited by the use of a glycogenolytic agent, adrenalin, and facilitated by histamine. A "true" hyperglycemia does not develop, however, as shown by the Hagerdorn-Jensen method. A similar situation has been reported for galactose cataracts (10). The high Folin-Malmros values may indicate a marked increase in the intermediate products of sugar metabolism, since this method is sensitive to non-glucose reducing substances. The significant increase in the incidence of cataract for fasted mice and the fact that histamine may start a demobilization of glycogen (15) and the development of an acidosis also point to acidosis as a possible etiological agent, but no consistent correlation with the incidence of cataract has been proved (cf. 14). The failure to induce opacities in mice after gynergen treatment, which prevents the adrenalin hyperglycemic response and the circulatory collapse, signifies nevertheless the essentiality of carbohydrate participation in the process of opacification.

A second complex of factors, namely, circulatory embarrassment and transfer of fluid to tissues, is according to several authors (9, 14, 16) the fundamental disturbance in diabetic states accounting for the hydrated state of the lens. The sequence of events between the systemic and ocular changes and the dominating extrinsic or intrinsic factors initiating imbibition of water by the lens are not yet clearly established, although the immediate osmotic imbalance and altered permeability may be conditioned by a plethora of carbohydrate metabolites (16), and certain intrinsic physico-chemical changes have been shown to parallel the cataractous process (cf. 5, 12).

Impairment of the circulation and disturbed fluid balance are predominant reactions in adrenalin intoxication (2, 8); and the marked vasodilatation of the uveal tract, strikingly visible in the eyes of albino mice, indicates that the ocular tissues are no exception to the systemic reaction. Furthermore, cataracts appear with the signs of anhydremia, and clear as circulatory improvement and diuresis begin. Hence the effect of histamine in increasing the incidence of opacities may well be due to an intensification of this second factor by its characteristic action on capillary dilatation and permeability, in addition to its possible effect on the carbohydrate cycle. That a combination of acute carbohydrate disturbance with the circulatory changes is essential for opacification is shown by the fact that histamine alone, or in amounts which offset the predominance of the adrenalin effects, leads to no opacification of the lens as the shock syndrome develops. Otherwise, rapid transitory opacities should be induced during the developmental stages of histamine shock, or their incidence should be increased rather than decreased as the ratio of histamine to adrenalin exceeds 25:1 (see table). Therefore it appears that conditions

which set up an acute glycogenolytic reaction (e.g., adrenalin intoxication), but not necessarily simple hyperglycemia, can lead to temporary changes in the stability of the lens and its capsule if there is an impairment of the circulation sufficient to interfere with complete oxidation of the products of carbohydrate breakdown and/or osmotic adjustments. A condition which aggravates either factor (or both, e.g., histamine) can facilitate the lens changes, although it may not be able to initiate them when acting alone.

SUMMARY

1. Reversible subcapsular lens opacities are produced in mice after single intraperitoneal injections of toxic but non-fatal amounts of adrenalin.

2. Moderate doses of histamine administered a few minutes before the injection of adrenalin increase the incidence of opacities and reduce the mortality of lethal doses of adrenalin.

3. The process of opacification appears to be related to some aspect of the carbohydrate disturbance (other than a simple elevation of the circulating glucose) and to the concomitant temporary circulatory impairment.

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A COMPARISON OF THE EFFECTS OF COLD AND THRYRO-TROPIC HORMONE ON THE THYROID GLAND

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Refrigeration of rats increases the activity of the thyroid gland. This fact has been experimentally shown by several investigators. Seidell and Fenger (1) in 1912 analyzed the iodine content of thyroid glands during summer and winter. They found evidence of iodine storage during summer and depletion in winter. Mills (2) in 1918 observed increased thyroid activity in rabbits, guinea pigs and cats after exposure to low environmental temperatures. Cramer (3) in 1920 reported observations on rats and mice after refrigeration and concluded that the low temperature produced experimental hyperthyroidism. Riddle (4) in 1927 reported that the thyroid gland of the pigeon is more active in winter than in summer. Benedict and McLeod (5) found higher oxygen consumption in animals living in the cold than in animals at room temperature. Mason (6) corroborated Benedict and McLeod's results on human subjects living in different climates. Kenyon (7) reported thyroid hypertrophy as a result of refrigeration of his animals. Kuschinsky (8), later in 1933, and Woitkewitsch (9) in 1935 confirmed Kenyon's work; and Wolf and Greep (10) reported similar results in 1937. Ring (11, 12) showed that the body temperature of animals increased when they were exposed to cold and that to demonstrate hypertrophy of the thyroid, a refrigeration period of three weeks was necessary. Uotilla (13, 14, 15, 16) has applied the cold-stimulus mechanism recently in determining the rôle of the sympathetics, anterior pituitary and hypothalamus in thyroid function, and has measured the increase in height of the thyroid epithelium. Other investigators have used various methods to determine the changes in the thyroid, namely, oxygen consumption, iodine storage, gross estimation of a microscopic picture, and weight gain of the glands.

METHOD. We applied the method of Starr and Rawson (17) to the assay of our specimens. We believe this is a more strictly quantitative procedure than any of the methods cited above. Tissues were removed from the animals and preserved in formalin fixative for 24 hours. They were then prepared for histological examination. The sections were examined under low power; the mean cell height was then measured under oil im-

mersion. The microscope was equipped with an eschelon ocular micrometer (Leitz) calibrated in micra. In measuring the cell height each acinus is observed. The average cell height is judged by eye and measured by the ocular micrometer. This measurement is recorded. Two hundred such acini are measured and the arithmetical mean cell height (M.C.H.) is determined.

Rats were exposed to the temperature of an ice refrigerator which was equipped with a double insulating side panel of glass, allowing a full view of the ice and cold chamber in which the rats were caged. The refrigerator was placed in a bright room. Air circulation was automatic by means of convection currents. The ice was placed in a chamber above the rat cages. A pipe equipped with a loose-fitting connection in which small holes were punched to allow correct exchange of air, drained the ice-chamber. The temperature of the refrigerator ranged from 12° to 17°C. Our refrigerator was never colder than 12°C., whereas the temperature of an electric refrigerator such as was used by former investigators, is maintained near freezing. We believe this temperature difference to be of great importance. The conditions of our experiment give the thyroid a moderate stimulus, producing gradual changes that can be measured. The relatively greater coldness of the electric condensing refrigerator produces violent changes in the thyroid. The animal, especially if handicapped by a complicating operation, can not survive more than a few days. The diet upon which the rats throve was a mixture of milk powder 4 parts, whole wheat flour 4 parts, and an equal amount of calcium carbonate and uniodized sodium chloride $\frac{1}{4}$ part. The time of cold exposure varied from 3 to 56 days. At the end of this period the rats were weighed, killed with ether inhalation and the thyroid glands removed.

Table 1 is a compilation of mean cell heights measured from the thyroids taken from control rats and rats which were refrigerated for varying periods of time, namely, 3, 7, 14, 18, 21, 28, 35, 45 and 56 days. The animals maintained at room temperature had an average cell height of 5.28 micra. Refrigeration for three days produced a limited but definite hypertrophy, the mean cell height for 10 animals averaging 5.90 micra. Refrigeration for 11 more days showed no further effect on the cell height which at that time (14 days) averaged only 5.87 micra, the average being calculated from measurements on eight rats. On the 18th day of refrigeration the cell height averaged from 7 animals was 7.95 micra. This is a marked secondary hypertrophy, which remains more or less stationary until the 45th day when the reaction is at a maximum, with six animals averaging a cell height of 9.08 micra. Between the 45th and 56th days the average cell height dropped from 9.08 micra at 45 days to 6.23 micra at 56 days. Seven rats were included in the 56 day series. This level at 56 days closely approximates the hypertrophy seen after three days of cold exposure.

The colloid gradually disappears until the 18th day when all that remains is a fine reticulum which in most cases continues in this state until after the 45th day when the colloid begins to reappear. After refrigeration for 56 days involution with colloid storage is greater in some animals than in others. The measurements of these changes are given in table 1 and illustrated in figures 1 to 6—photomicrographs of the glands of animals killed after varying periods of time; figure 1 is a section of a room temperature control rat; figure 2 is a section of the gland of a rat refrigerated for 3 days; figures 3 to 6 are pictures of glands autopsied at 14, 21, 45 and 56 days.

To investigate the rôle of iodine in this cycle of events we refrigerated 17 rats for 21 days. To one group of 3 rats, after preliminary ice-box treatment of 24 days' duration, we administered iodine daily in doses of 10,000 gamma, for three days in the form of potassium iodide solution in-

TABLE 1

Rat thyroid mean cell height in micra after increasing duration of refrigeration at 15°C.

REFRIGERATION TIME	NUMBER	RANGE	AVERAGE MEAN	STATISTICAL MEAN	CRITICAL RATIO
days					
Room temp.	11	4.97– 5.98	5.28	5.323	
3	10	4.80– 6.60	5.90	5.825	11.1
7	6	5.57– 6.43	6.08	5.908	11.2
14	8	5.42– 6.56	5.87	5.891	11.7
18	7	6.64– 9.47	7.95	8.091	52.1
21	11	6.61– 9.36	7.89	7.964	52.4
28	8	5.26– 9.16	7.45	7.473	33.9
35	5	7.11– 8.35	7.80	7.797	39.2
45	6	8.0 –10.28	9.08	9.016	60.6
56	7	5.34– 7.10	6.23	6.224	18.8

traperitoneally, and then killed the rats. The cell heights averaged 8.81 micra. Another group of 3 rats was refrigerated for 21 days and then injected with potassium iodide in daily doses of 2000 gamma for a period of 12 days. They were autopsied on the 33rd day. The mean cell heights of these animals averaged 5.73 micra. Still another series of 9 rats was refrigerated for 21 days. Injections of sodium iodide solution of different concentrations were begun on the third day of refrigeration; three of the group were injected intraperitoneally with 10 gamma daily doses of sodium iodide; three received 1000 gamma of sodium iodide intraperitoneally per day; and the third group of three rats was given daily injections intraperitoneally of 10,000 gamma. The concentrations were such that each animal received 1 cc. per day. On the 21st day the rats were autopsied, 21 days' refrigeration being sufficient without iodine supplements to cause the secondary hypertrophy. The average thyroid mean cell height of the

animals of this series receiving 10 gamma of potassium iodide was 6.85 micra; that of those receiving 1000 gamma was 5.93 micra; and that of those receiving 10,000 gamma of sodium iodide was 5.18 micra.

A further series of 8 rats was injected with sodium iodide. The entire series was given pre-refrigeration treatment with sodium iodide, three rats receiving 10 gamma per day; two, 1000 gamma daily; and three receiving 10,000 gamma per day. The injections were continued for 26
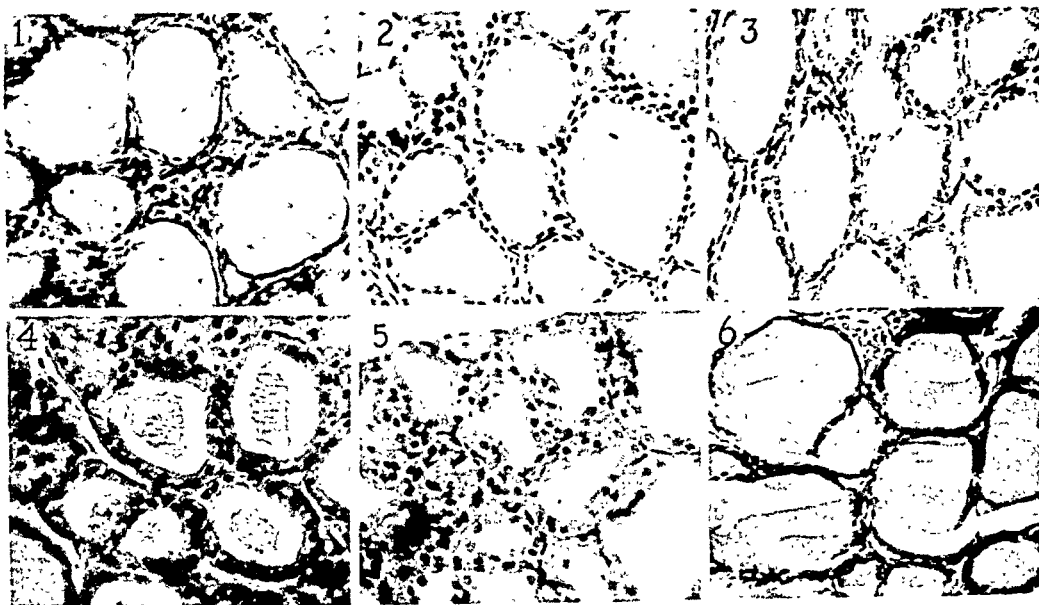
Figs. 1 to 6. Photomicrographs showing histological changes in the thyroid produced by continuous refrigeration at 12 to 17°C.

Fig. 1. Control.

Fig. 2. Three days' refrigeration, showing some loss of colloid and a small amount of hypertrophy of the acinar cells.

Fig. 3. Fourteen days' refrigeration: The cells remain the same height but the colloid has been lost from the acinar lumen.

Fig. 4. Twenty-one days' refrigeration: Colloid reticulum only remains; cells definitely more hypertrophic.

Fig. 5. Forty-five days' refrigeration: Cells markedly hypertrophic.

Fig. 6. Fifty-six days' refrigeration: Involution of hypertrophy; cells are lower in height; colloid storage has occurred.

days. On the fifth day of treatment the animals were placed in the refrigerator where they remained for 21 days. At the end of this time they were killed and the thyroid studied. The mean cell height of the animals receiving 10 gamma was 6.26 micra; that of those receiving 1000 gamma averaged 6.85 micra; and that of those receiving 10,000 gamma was 6.22 micra (see table 2). All of these animals were refrigerated an adequate length of time to allow a maximum hypertrophy of the acinar cells, that is, a mean cell height of 7.95 micra, as noted after the 18th day of refrigera-

tion.   In all but three of these animals the thyroid epithelium was lowered at least to the level noted at three days of refrigeration; the three which remained high were injected only three days with a dosage of 10,000 gamma of potassium iodide, after secondary hypertrophy had been produced with iodine.   This time factor probably played a dominant rôle in the submaximal response to the iodine administered.   In the group of animals in which the cell height was markedly reduced, three showed reduction to a level the same as that of the room temperature control rats; the remaining 17 rats (73.9 per cent) showed cell counts approximating the level of the primary slight hypertrophy noted after three days' refrigeration.

These experiments suggest that even a large dosage of iodine over a short period of time is insufficient to lower the cell height in a thyroid gland which histologically shows the picture of secondary hypertrophy.   They further suggest that the small amount of hypertrophy noted from 3 to 14

TABLE ·2

The effect of iodine on rat thyroid mean cell height during refrigeration

GROUP	NUMBER OF ANIMALS	REFRIGERATION TIME	IODINE DOSAGE	NUMBER OF DAYS TREATED	MEAN CELL HEIGHT
		days	*gamma*		*micra*
I	3	27	10,000	3	8.81
	3	33	20,000	12	5.73
II	3	21	10	19	6.85
	3	21	1,000	19	5.93
	3	21	10,000	19	5.18
III	3	21	10	26	6.26
	2	21	1,000	26	5.85
	3	21	10,000	26	6.22

days of refrigeration is not due to iodine deficiency but to a functional activity of the thyroid gland in response to adequate stimulus; but that the further increase in cell height seen after 18 days is due to a deficiency in the supply of iodine to the animal.   The increased cell height before depletion of colloid, that is, from 3 to 14 days, is an indication of an increased rate of thyroid hormone formation in response to need created by refrigeration.   The additional secondary hypertrophy after colloid depletion (from 18 to 45 days) is not an index of a still further increased rate of thyroid hormone production since it can be prevented by providing iodine.   The cold environment at 18 days is the same as that at 14 days.   There is, therefore, no greater need for thyroid hormone at 18 or 45 days than there was from 3 to 14 days.

One would expect the same type of early plateau in animals injected with increasing amounts of thyrotropic hormone.   In examining Starr and Rawson's curve (17), made from the measurements of guinea pigs given

Antuitrin-T (Parke, Davis),[1] one dose per day for three days at room temperature, the animals being killed on the fourth day, an increase in cell height is observed as the dosage is increased (fig. 7).

An Antuitrin-T dosage of 0.0025 cc. increased the mean cell height 0.69 micra over the control level of 3.93 micra. An increase in dosage of 0.0025 cc. to 0.005 cc. increases the cell height by an additional 0.46 micra. This is double the original dose, or a 100 per cent increment. Now, if the dosage is again doubled, to make the daily injection 0.01 cc. of Antuitrin-T, the actual increase in injected hormone is 0.005 cc. or twice that of the former increment, but the reaction is still only sufficient to increase the mean cell height another 0.52 micra. A daily dosage increase from 0.01 to 0.02 cc. is an increase of 0.01 cc. per injection and is twice the increment of the

TABLE 3

Guinea pig thyroid mean cell height with increasing dosage of thyrotropic extract acting for three days

ANTUITRIN-T DOSAGE	DOSAGE INCREMENT	CELL HEIGHT	CELL HEIGHT INCREASE
cc.		*micra*	
Control		3.93	
0.0025	0.0025	4.62	+0.69
0.005	0.0025	5.08	+0.46
0.01	0.005	5.60	+0.52
0.02	0.01	6.09	+0.49
0.03	0.01	7.96	+1.87
0.04	0.01	8.50	+0.54
0.05	0.01	7.87	−0.63
0.085	0.035	8.28	+0.41
0.1	0.015	8.02	−0.26
0.5	0.400	8.23	+0.21

former case. This dosage of 0.02 cc. increases the mean cell height an additional 0.49 micra. Then, if the daily dosage is increased from 0.02 to 0.03 cc., an increment of the same amount as between 0.01 and 0.02 cc., the cell height increase is 1.87 micra or nearly four times the increase seen with the same increments but small total dose as reported above. Further, increase of daily dosage above 0.03 cc. results in no further increase in cell height.

The interpretation of this series of experiments was that as the dosage of Antuitrin-T was increased, the mean cell height increased steadily until a maximum cell height was reached, at which time an excess of Antuitrin-T failed to produce further increase. With our interpretation that the

[1] Generously provided by Dr. E. A. Sharp of Parke, Davis and Company.

greater hypertrophy is an index of iodine depletion, we may analyze the curve of Starr and Rawson and find that if a line is drawn touching the points plotted for the mean cell height levels, the curve resembles that drawn from glands of refrigerated animals (fig. 7). Thus, there is a slow increase in cell height until a dosage of somewhere between 0.02 and 0.03 cc. of Antuitrin-T is reached. Doses larger than 0.02 cc. of Antuitrin-T daily cause a response out of proportion to the increase in dosage. The histological picture is one of gradual colloid depletion until after the dosage
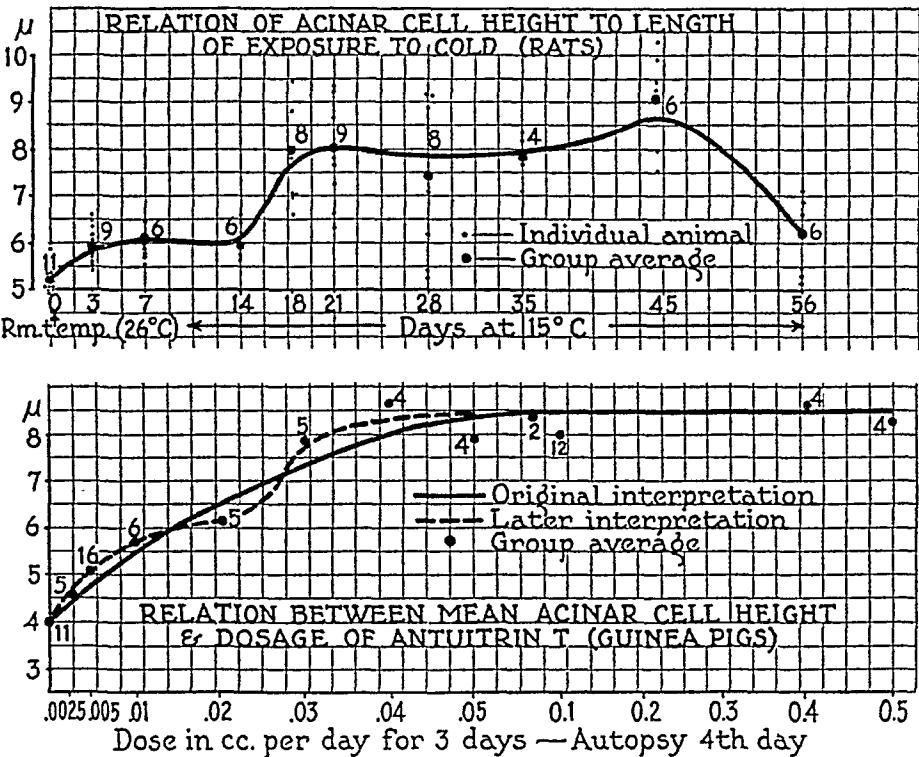
Fig. 7. Curves drawn from thyroid mean cell height measurements of rats after refrigeration and guinea pigs after three daily injections of Antuitrin-T. Figures on Antuitrin-T curve indicate number of animals used.

of 0.02 cc. Antuitrin-T is reached. A dosage of 0.03 cc. of Antuitrin-T causes complete absence of colloid with very high acinar cells.

This analysis suggests that the cell heights of 3.93 micra to 6.09 micra are an evidence of functional activity, while the ultimate cell heights, 7.96 to 8.50 micra, are evidence of thyroid response in the presence of colloid depletion. The period of observation in these assays, 96 hours, allows depletion to occur in response to the first and/or second injections. When these are of the critical amount found to be more than 0.02 cc. Antuitrin-T the third injection produces the excessive secondary response.

SUMMARY

Eleven rats were killed at different times during the year and a normal cell height of the thyroid epithelium of 5.28 micra was found. The mean cell heights varied from 4.97 to 5.98 micra.

In a series of 24 rats exposed to cold over periods of time ranging from 3 to 14 days, the average cell height of the thyroid glands was 5.95 micra, which is definite but limited hypertrophy of the acinar cells.

After 18 days in the ice-box a group of 7 rats was killed, and the average cell height of this group measured 7.95 micra.

Thirty-seven rats killed between 18 and 45 days showed an average cell height of 8.03 micra.

Seven rats killed after 56 days in the ice-box showed an average cell height of 6.23 micra, which is approximately the level of the small amount of hypertrophy noted after 3 days in the cold environment.

Iodine in amounts as small as 10 gamma per day, while rats are being refrigerated, is sufficient to limit hypertrophy to an amount equal to that noted after 3 days' refrigeration without iodine, but it does not prevent it. Larger doses, up to 10,000 gamma per day for 26 days, do not materially change this cell height but prevent the secondary hypertrophy resulting from 18 to 45 day terms of exposure to this cold environment.

Analysis of the thyrotropic hormone assay curve of Starr and Rawson indicates primary and secondary levels of epithelial hypertrophy, probably indicative of colloid depletion during the three day period of assay.

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MODIFICATION OF THE ELECTRICAL POTENTIAL OF FROG SKIN BY ACETYLCHOLINE

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Fluctuations in the electrical potential of the skin of the frog and other animals produced by stimulation of the cutaneous nerves have been described by several investigators (for references to the early work of Hermann, Engelmann and others cf. Orbeli, 1910). Schwarz (1915) has related these phenomena to features of the psychogalvanic reflex. Motokawa (1939) has recently reported that the potential of frog skin is modified by acetylcholine. This paper came to my attention while the present experiments were in progress. Motokawa, however, did not use a recording potentiometer.

METHODS. For the study of the effects of nerve stimulation a dorsal skin with cutaneous nerves and spinal cord attached was tied inside out on a vertical glass tube (1.4 cm. in diameter) containing Ringer's solution mounted in a beaker of Ringer's. One Ag-AgCl electrode made contact with the negative morphological outside of the skin through the Ringer's in a side arm of the vertical tube. The other Ag-AgCl electrode made contact with the positive morphological inside of the skin through the outer beaker of Ringer's. The electrodes were connected to a Micromax recording potentiometer or a type K potentiometer. The spinal cord was gently lifted by a thread out of the solution and the nerves running to the morphological inside of the skin (inverted on the tube) were stimulated by Ag-AgCl electrodes with a tetanizing current from a Harvard coil (1 dry cell, coil distance usually zero). For the acetylcholine experiments skins were tied on the blunt end of medicine droppers mounted on no. 4 rubber stoppers. The medicine dropper was filled with Ringer's and clamped vertically so that the skin was immersed in a small beaker of Ringer's solution with or without acetylcholine. These simple skin holders permitted testing a large number of skins in the same solution of acetylcholine. A few skins were treated in the glass skin holder previously described (cf. Barnes, 1939a, fig. 1) through which oxygen was bubbled. In all cases the Ringer's was changed just before treatment with acetylcholine to prevent stimulation by fresh medium. The concentration of acetylcholine (0.66 to 1.0 per cent) is recorded in table 1.

The solution was made up immediately before use. The pH of the solutions was taken with a glass electrode to detect any high alkalinity which is known to hydrolyse acetylcholine even in the absence of esterase (cf. Barbour and Dickerson, 1939). The oxygen consumption of skins treated with acetylcholine was measured in Fenn respirometers.

RESULTS. Single induction shocks applied to the nerves had slight effects on the skin potential but interrupted currents lasting from 1 to 60 seconds produced large changes in the skin potential (see fig. 1A). With
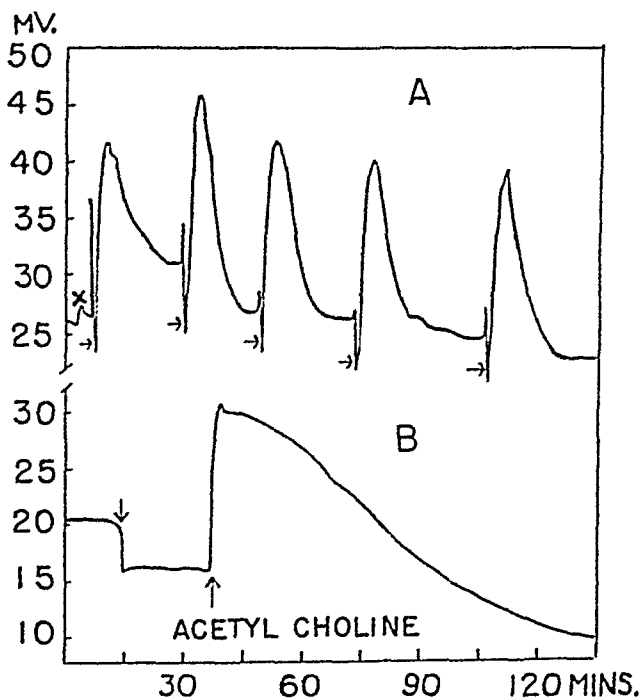
Fig. 1. *A*. The effects of stimulating nerves to the skin on skin potential of the frog. Ordinates: e. m. f. in millivolts (outside surface of skin is negative). Abscissae: time in fifteen-minute intervals. Micromax recorded. The five large peaks follow a 5, 10, 15, 20 and 25 second tetanizing current. The first small wave (marked *x*) following a single break shock. Arrows indicate end of each nerve stimulation. Harvard inductorum: coil distance 0; one dry cell.

B. Effect of 0.70 per cent acetylcholine in Ringer's. At first arrow Ringer's was changed for fresh ordinary Ringer's to eliminate effect of fresh medium.

high potentials (above 50 mv.) the potential usually fell, but with lower potentials the potential frequently increased. The magnitude of the potential change varied from one preparation to another. It is important to note, however, that the results for a given skin were usually constant (see fig. 1A). It was noted that the potential changes always outlasted the stimulus. In many cases the large electrical wave did not begin until the stimulus had ceased (see fig. 1A).

Acetylcholine in a concentration of 0.10 per cent increased the skin

potential when applied to the morphological outside surface (average increase 61.2 per cent, 6 cases) and depressed the potential when applied to the morphological inside surface (average decrease 13.1 per cent, 5 cases). In a concentration of 0.66 per cent acetylcholine on the outside of the skin increased potential 127.7 per cent (6 cases) and on the inside depressed potential 62.0 per cent (5 cases). With 1.0 per cent acetylcholine the average increase after outside treatment was 94.9 per cent (5 cases) and the average decrease after inside application was 60.9 per cent (6 cases). A number of typical experiments are recorded in table 1.

TABLE 1

Effect of acetylcholine on the potential of frog skin

Two preparations from each frog. Numbers 1 and 2 from frog 1; 2 and 3 from frog 2, etc. Frogs weighed 31.0 to 42.5 grams. All males except frogs 1 and 2. 0 = outside surface treated; i = inside surface treated; a = anterior region of belly; p = posterior region of belly. Temperature taken at every reading, 22–23° C. pH of solutions 7.03–7.70.

NUM-BER	DATE	SURFACE TREATED	E. M. F. BEFORE	CONCEN-TRATION	E. M. F. AFTER	TIME OF ACTION	REMARKS
			mv.	*per cent*	*mv.*	*minutes*	
1	April 6	o. a.	16.3	0.10	19.3	6	18.4% rise
2	April 6	i. p.	37.0	0.10	30.0	5	18.9% fall
3	April 6	o. a.	5.7	0.10	17.0	4	198.2% rise
4	April 6	i. p.	65.6	0.10	58.0	9	11.5% fall
5	April 4	o. a.	2.0	0.66	5.8	1	190% rise
6	April 4	o. p.	8.3	0.66	13.8	5	65.2% rise
7	April 4	i. p.	39.3	0.66	33.7	1	14.2% fall
8	April 4	i. p.	51.0	0.66	29.0	8	43.1% fall
9	April 5	o. a.	11.3	1.0	21.0	4	85.8% rise
10	April 5	i. p.	23.6	1.0	7.0	1	70.3% fall
11	April 5	o. a.	9.0	1.0	25.9	11	187.7% rise
12	April 5	i. p.	35.0	1.0	12.4	8	64.5% fall

Although there is no *a priori* reason that acetylcholine should affect the metabolism of the skin, the O_2 uptake was measured owing to the important though indirect linkage between oxidation and skin potential (for references cf. Barnes, 1939b). The skin from the ventral surface of a frog was divided into three parts and the pieces placed in ordinary Ringer's solution in three Fenn respirometers. The O_2 uptake was measured for a preliminary interval of about one hour. Acetylcholine Ringer's was then introduced into two flasks and fresh Ringer's into the third as a control. The O_2 uptake was again measured for an hour or more. The concentrations of acetylcholine used were 0.066 per cent to 1.0 per cent but no appreciable effect on oxygen consumption was detected. The O_2 uptake of skins in acetylcholine Ringer's fell—18.2 per cent during the

run (8 cases), while the O_2 uptake of control skins in fresh ordinary Ringer's fell—24.4 per cent (4 cases). This represents a possible 6.2 per cent increase in respiration produced by acetylcholine but is probably not significant.

DISCUSSION. The delayed effect on potential of nerve stimulation suggests chemical mediation of the nerve impulse to the skin. The stimulating action of acetylcholine on the outside surface of the skin also supports this hypothesis. "The increase in the output of electrical energy produced by acetylcholine is of interest in connection with the problem of potentials arising in muscle and ganglia through the chemical mediation of the nervous impulse. The action of acetylcholine on skin potential, however, probably involves mechanisms essentially distinct from synaptic and neuro-muscular transmission. The persistence of the electrical wave in skin indicates that the choline esterase is not very active in this tissue. It is suggested that the frog skin should prove a particularly useful preparation for the study of the mode of action of acetylcholine on bioelectrical potential. It is possible that acetylcholine mobilizes electrically active ions such as K or Na in the skin, for there is evidence that skin potential is produced by ionic diffusion (cf. Amberson, 1936; Dean, 1939; Barnes, 1940). It is well known that acetylcholine is associated with potassium metabolism (Eccles, 1936). Moreover, Cicardo and Moglia (1940) have shown that acetylcholine liberates potassium from muscle.

The depressing of potential by acetylcholine on the inside surface of the skin is difficult to explain but is not very important for the present discussion, for it is known that the electrical potential is located on the outside surface (cf. Barnes, 1940). Moreover, the skins treated on the inside were reversed on the holder. It was shown (Barnes, 1939a) that contact between the outside surface and the glass holder raises the potential as can be seen in table 1 in which the E. M. F. of skins from the same frog are recorded in normal and reversed orientation. Skins marked "i" in table 1 were treated on the inside and consequently were tied inside out on the holder. These unusually high potentials are probably unstable. The depression may be the result of the high concentration of acetylcholine used in the experiments. Thus 0.66 per cent produced a depression of 77.7 per cent, while 0.10 per cent depressed only 13.1 per cent. The inhibiting action of acetylcholine is well known to depend on concentration in other tissues.

The respirometer experiments indicate that acetylcholine is not altering potential through oxidative mechanisms of the skin. Fifteen minutes elapsed between the introduction of acetylcholine into the flask and the next reading of O_2 uptake. To detect any initial effect which might have been missed during this interval, five experiments were run with flasks having sidearms for tipping in the reagent. The average O_2 uptake

showed an increase of 1.5 per cent over the entire runs and an average of 14.9 per cent increase for the interval 15 to 19 minutes immediately after tipping. (The stop cocks were closed for about 3 minutes during the operation of tipping the respirometer.) The slight increase in O_2 uptake on tipping is probably the result of adding fresh Ringer's solution. The concentration of acetylcholine in these five runs was 0.20 to 0.32 per cent.

SUMMARY

1. Stimulation of the nerves of frog skin preparations with a tetanizing current produces large changes in potential of the skin which always outlast the stimulus.

2. Acetylcholine produces a rise in the electrical potential of frog skin when applied to the outside surface.

3. Acetylcholine has little or no effect on the oxygen consumption of the skin.

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ON THE MECHANISM OF THE DEPRESSION OF THE SERUM POTASSIUM LEVEL BY EPINEPHRINE

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It has been shown by Bachromejeu (1), D'Silva (2) and others that intravenous administration of epinephrine results in a brief preliminary increase in serum potassium which is followed by a prolonged fall to below control levels. Insulin also causes a marked lowering of the serum potassium level (Briggs et al. (3), Kerr (4)); an effect which is independent of the presence of the adrenal medulla (5). The possibility that the ultimate decrease in serum potassium following epinephrine administration is at least in part a result of mobilization of insulin has been investigated by D'Silva (2). D'Silva found that pancreatectomy reduced the lowering effect of epinephrine on serum potassium but did not abolish it. In my experience in this connection, the result of pancreatectomy on the subsequent course of epinephrine is unpredictable. Apparently, mechanisms in addition to insulin mobilization are involved.

Fenn and Cobb (6) have shown that a decrease in the plasma potassium concentration can be accomplished by increasing the hydrogen ion concentration within the cell (frog muscle). Of interest in this connection is the observation by Pulver (7) that if glucose be added to a suspension of yeast cells, the potassium concentration of the solution surrounding the cells is markedly lowered as fermentation proceeds. After fermentation is essentially complete the potassium concentration slowly returns to the control level. Simultaneously with the onset of fermentation, a marked, irreversible drop in pH occurs. It is conceivable that potassium enters the cell in response to the pH gradient set up by fermentation and slowly returns with establishment of equilibrium conditions when fermentation is over.

Although it is well known that epinephrine increases the lactic acid content of the blood (8, 9) and lowers the alkali reserve (10), data as to its effect on blood pH are rather incomplete. The effect of epinephrine on blood pH has therefore been investigated in the hope that it might cast some light on the serum potassium changes.

EXPERIMENTAL. All serum potassium and pH measurements were

made on arterial blood. Whenever anesthetics were administered, a period of at least one hour was allowed to elapse for the stabilization of serum potassium and blood pH prior to the epinephrine infusion. Control pH measurements were made at 10 to 15 minute intervals prior to the epinephrine or insulin administration until readings that were constant or varied by no more than 0.01 pH were obtained.

The effect of subcutaneously administered epinephrine (0.5 mgm. per kgm. of body weight) was investigated on unanesthetized dogs. The effect of intravenously infused epinephrine (0.005 mgm. per kgm. of body weight per minute) was investigated on a, cats anesthetized with Dial;[1] b, eviscerated, hepatectomized cats under Dial anesthesia, and c, cats anesthetized with Dial and receiving constant artificial ventilation. For the cats in part c blood pH was determined prior to the application of artificial ventilation and subsequently the artificial ventilation was adjusted to give control blood pH readings just slightly above (within 0.03 pH) that maintained by the cat's own respiration. The effect of sub-

TABLE 1

Effect of epinephrine (0.5 mgm. per kgm. body weight, subcutaneously) on blood pH and serum potassium of unanesthetized dogs

| | BLOOD pH AND SERUM K CHANGE AT VARYING INTERVALS AFTER EPINEPHRINE EXPRESSED AS AVERAGES OF RESULTS ON 3 DOGS | | | |
	15 minutes	30 minutes	60 minutes	120 minutes
pH..	−0.01	−0.02	−0.07	−0.12
Serum K in millimols per liter..............	−0.43	−0.69	−1.12	−1.28

cutaneously administered insulin (0.5 unit per kgm. of body weight) was investigated on unanesthetized dogs.

All pH measurements were made with the glass electrode. Potassium determinations were made on serum by the method of Kramer and Tisdall (11).

RESULTS AND DISCUSSION. The effect of subcutaneously administered epinephrine on blood pH and serum potassium of unanesthetized dogs is shown in table 1. A simultaneous lowering of both serum potassium and blood pH occurred. However, no strict proportionality between the two is evident.

Because of possible variations in the rate of absorption of subcutaneously injected epinephrine and the desirability of having more exact information on the immediate effects of epinephrine, a series of experiments was performed on anesthetized cats into which epinephrine was infused intravenously at a constant rate for 30 minutes. The results of these experi-

ments are shown in table 2.   Two types of pH changes were observed; and, for this reason, the animals have been divided into two groups.   In 5 of the 8 cats used, blood pH fell promptly reaching a new level in approximately 20 minutes, followed by a return to near control levels within 30 minutes after cessation of the infusion.   The other 3 cats showed a preliminary rise in pH followed by the fall to below control levels.

The serum potassium changes showed no significant variations between the two groups and have therefore been included as representative of the 8 cats.   The initial increase due to release of potassium from the liver is present.   Whether the initial rise in pH occasionally observed bears any relation to the mechanism by which epinephrine effects a release of potassium from the liver is not known and the present technic is not suitable for further inquiry along this line.   Following the initial rise in serum potassium the fall to below control levels occurred.   An additional, and

TABLE 2

Effect of epinephrine (0.005 mgm. per kgm. per minute, intravenously) on blood pH and serum potassium of anesthetized cats

	BLOOD pH AND SERUM K CHANGE AT VARYING INTERVALS DURING EPINEPHRINE INFUSION EXPRESSED AS AVERAGES					BLOOD pH AND SERUM K CHANGE AT INTERVALS FOLLOWING CESSATION OF EPINEPHRINE INFUSION EXPRESSED AS AVERAGES		
	2 minutes	7 minutes	12 minutes	20 minutes	30 minutes	10 minutes	20 minutes	30 minutes
pH, 5 animals........	−0.03	−0.09	−0.12	−0.13	−0.13	−0.07	−0.04	−0.02
pH, 3 animals........	+0.04	+0.01	−0.06	−0.08	−0.09	−0.06	−0.03	−0.01
Serum K in millimols per liter..........	+4.60	+1.53	+0.01	−1.02	−1.03	−3.07	−1.79	−1.41

to the author's knowledge a hitherto unrecorded, feature occurred on cessation of the epinephrine infusion.   Instead of the serum potassium immediately beginning to return to control levels, there resulted a sharp secondary decrease followed by the process of gradual recovery. .

As stated above, an initial effect of epinephrine is to bring about a release of potassium from the liver.   Apparently, at the end of the epinephrine infusion the liver rapidly recovers its potassium stores at the expense of the extracellular fluids.   To test this, the effect of epinephrine on the serum potassium of eviscerated, hepatectomized cats was investigated. Results of experiments on 3 cats are presented in table 3.

In the absence of the liver, the initial rise in serum potassium does not occur.   The fall in serum potassium begins immediately; and, on cessation of epinephrine administration, the return of serum potassium toward control levels is not interrupted by a secondary fall.   The rapidity with which the liver, deprived of some of its potassium, picks up potassium from

the extracellular fluids is an additional factor in the ultimate fall in serum potassium under the influence of epinephrine.   Incidentally, this also may explain why Heppel (12) found no significant decrease in liver potassium in his potassium deprived rats.

It will be noted in table 3 that during epinephrine infusion blood pH fell promptly to a lower level but differed from the experiments on the intact cat in that on cessation of the infusion recovery was much more prompt.   For some unknown reason these animals showed a marked

TABLE 3

Effect of epinephrine (0.005 mgm. per kgm. body weight per minute intravenously) on blood pH and serum potassium of anesthetized, eviscerated, hepatectomized cats

	BLOOD pH AND SERUM K CHANGE AT VARYING INTERVALS DURING EPINEPHRINE INFUSION EXPRESSED AS AVERAGES				BLOOD pH AND SERUM K CHANGE AT INTERVALS FOLLOWING CESSATION OF EPINEPHRINE INFUSION EXPRESSED AS AVERAGES		
	2 minutes	7 minutes	12 minutes	20 minutes	10 minutes	20 minutes	30 minutes
pH....................	−0.04	−0.07	−0.09	−0.09	+0.04		
Serum K in millimols per liter...........	−0.13	−0.26	−1.12	−1.28	−0.85	−0.54	−0.33

TABLE 4

Effect of epinephrine (0.005 mgm. per kgm. per minute, intravenously) on blood pH of anesthetized cats receiving constant artificial ventilation

	BLOOD pH CHANGE AT VARYING INTERVALS DURING EPINEPHRINE INFUSION EXPRESSED AS AVERAGES					BLOOD pH CHANGE AT VARYING INTERVALS FOLLOWING CESSATION OF EPINEPHRINE INFUSION EXPRESSED AS AVERAGES		
	2 minutes	7 minutes	12 minutes	20 minutes	30 minutes	10 minutes	20 minutes	30 minutes
pH, 3 animals........	−0.03	−0.11	−0.14	−0.16	−0.17	−0.10	−0.06	−0.03
pH, 1 animal........	+0.02	−0.03	−0.05	−0.05		0.00		

acceleration in respiratory rate on cessation of the epinephrine infusion, whereas the intact cats did not.

To determine if variations in respiration might have a rôle in the observed blood pH changes following epinephrine, 4 cats were given constant artificial ventilation throughout the experiment.   The results are recorded in table 4.   In 3 of these animals blood pH fell promptly and markedly during the epinephrine infusion, while in one a slight rise in pH was followed by only a slight fall.   The degree and character of the pH changes in these experiments compare favorably with those observed in intact, anesthetized cats (table 2).   Apparently change in pulmonary ventilation is

not a causative factor in the blood pH changes during epinephrine infusion into intact, anesthetized cats and the pH changes probably have their origin within the cell.   Recent experiments by Griffith, Emery and Lockwood (13) lend further support to this assumption.   These workers found that epinephrine, in doses equivalent to those employed here, causes a small but definite increase in pulmonary ventilation.   This effect would be opposed to the blood pH decrease observed here.

Artificial ventilation experiments with eviscerated, hepatectomized cats were not successful.   Apparently this was due to lack of reserve blood supplies in these animals.   While it is possible to equilibrate fairly well their blood pH under artificial ventilation, withdrawal of quantities of blood for analysis in the absence of reserve supplies in itself alters the pH balance in a manner that cannot be compensated for in this type of experiment.

Although insulin has been previously reported as having no demonstrable effect on blood pH, in view of its pronounced effect on serum

TABLE 5

Effect of insulin (0.5 unit per kgm., sucutaneously) on blood pH and serum potassium of unanesthetized dogs

	BLOOD pH AND SERUM K CHANGE AT VARYING INTERVALS AFTER INSULIN EXPRESSED AS AVERAGES OF RESULTS ON 3 DOGS		
	30 minutes	60 minutes	120 minutes
pH.............................	+0.02	+0.02	0.00
Serum K in millimols per liter.....	−0.38	−0.67	−1.28

potassium, it was felt that this experiment might bear repetition.   Results on 3 dogs are recorded in table 5.   Serum potassium decreased markedly but no significant changes occurred in blood pH.   The mechanism by which insulin exerts its effect on serum potassium is not well known.   However, the simultaneous decrease that occurs in serum phosphate may have some bearing in this connection.

SUMMARY

The action of epinephrine in effecting a decrease in serum potassium cannot be completely explained as being a result of secondary mobilization of insulin.   It has been shown here that epinephrine causes a marked lowering in blood pH, an effect which very probably has its origin in an increase in the hydrogen ion concentration within the cell.   By analogy to Fenn and Cobb's findings (6) that increases in the hydrogen ion concentration within the cell lead to a lowering of the potassium concentration in the extracellular fluids, it is suggested that this may explain in part the decreases in serum potassium observed following epinephrine.

An additional factor in the ultimate fall in serum potassium is the rapidity with which the liver replenishes its stores of potassium at the expense of the extracellular fluids following cessation of epinephrine administration.

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THE EFFECT OF VARIOUS AGENTS ON THE BLOOD PRESSURE OF RENAL HYPERTENSIVE DOGS[1]

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Bilateral constriction of the renal arteries leads to a persistent hypertension in dogs (1) which in many respects resembles essential hypertension in man. The similarities include a normal cardiac output, blood volume, blood viscosity, and hematologic picture; normal blood chemistry and renal function tests except in the malignant phase; a failure to respond significantly to various operative procedures on the sympathetic nervous system; a reduced renal blood flow; an increased peripheral resistance; arteriolosclerosis in various organs; and an increased renin content of the kidneys (2, 3). We, therefore, studied a group of active principles and organ and plant extracts which have been reported to be of value in the treatment of essential hypertension to determine their effect on the blood pressures of renal hypertensive dogs. These included estrone, testosterone, and extracts of liver, pancreas, and garlic and parsley. Obviously, if the results with these substances resembled those obtained in essential hypertension, the findings would constitute further evidence for a parallelism between experimental renal hypertension and essential hypertension. On the other hand, a difference would be evidence against a similarity of the two conditions.

The pathogenesis of experimental renal ischemic hypertension still remains unsolved. Inconclusive evidence points to the release of a humoral agent from the ischemic kidney (2) but whether it acts directly or otherwise on the vessels to increase the peripheral resistance is not known. Consequently we studied the effect on the blood pressures of renal hypertensive dogs of a group of extracts prepared from organs definitely or possibly involved in the pathogenesis of experimental renal hypertension. The preparations included in this second group were renin, whole kidney, adrenal cortical extract, whole pituitary extract, and pituitrin.

During the course of the work an opportunity was afforded for observing the effect of distemper and of extensive cellulitis on the blood pressures of renal hypertensive dogs.

METHOD. Dogs weighing 9 to 12 kgm. were subjected to bilateral constriction of the renal arteries by the Goldblatt technique (1, 2) and the resulting hypertension was permitted to stabilize over a period of three or four months. In some cases further constriction of the arteries proved necessary in order to sustain the hypertension (2). Mean blood pressure readings by the method of Dameshek and Loman (4) were obtained from the femoral artery three times per week. Blood urea determinations and urinalyses were made when indicated. The study of each preparation involved a preliminary control period of one or two months during which the blood pressure of the animal was determined to be relatively constant. This was followed by an experimental period during which the preparation was administered daily in most cases for one month. Subsequently there was another control period of one or two months. Some of the animals were later used for the study of other active principles or extracts. Parallel observations were made on normal dogs with several of the substances.

RESULTS. *Group 1.* a. Estrone. Daily intramuscular injections of 0.1 mgm. (1000 i.u.) per kilo of estrone[2] into one hypertensive dog for one month had no significant effect on the blood pressure of the animal.

b. Testosterone. Daily intramuscular injections of 2.5 mgm. per kilo of testosterone[3] into one hypertensive dog for one month did not appreciably influence the pressure levels.

c. Liver extract. A peptone-containing aqueous extract of liver[4] said to contain none of the known depressor substances was tested in two hypertensive dogs. One of the dogs was given 2 cc. of the extract intravenously twice daily and intramuscularly once per day for one month. The other animal was subjected to the same regimen with twice the foregoing dosage. There was no significant change in the blood pressures of either dog as a result of the liver extract injections.

d. Pancreatic extract. A preparation containing desiccated pancreas and thyroid[5] in a ratio of 25:1 was administered orally to one hypertensive dog in a dosage of 0.3 gram daily for one month. The pressure levels of the animal were unaltered.

e. Garlic and parsley. A preparation of desiccated garlic and parsley was administered orally to one hypertensive dog in a dosage of approximately 2 grams daily for two months without any significant change in the blood pressure.

[2] Theelin, supplied by Parke, Davis and Company, Detroit, Michigan (Dr. Oliver Kamm).

[3] Perandren, supplied by Ciba Pharmaceutical Products, Summit, N. J. (Dr. R. MacBrayer).

[4] Supplied by The Armour Laboratories, Chicago, Illinois (Dr. E. F. Pike).

[5] Panlittol, supplied by The Armour Laboratories, Chicago, Illinois (Dr. E. F. Pike).

The results with this first group of hormones and extracts are summarized in table 1.

Group 2. a. Renin. One hypertensive and one normal dog were given daily intramuscular injections of dog renin (equivalent in acute pressor effect intravenously to approximately 4 units of pituitrin) for two and one-half months. A similar pair of dogs received daily intravenous injections of dog renin for one month in twice the foregoing dosage. The two hypertensive dogs showed average increases of 10 and 15 mm. of Hg

TABLE 1

The effect of various agents on the blood pressure of renal hypersensitive and normal dogs

EXPERIMENT NUMBER	ACTIVE PRINCIPLE OR EXTRACT	DAILY DOSAGE	ROUTE OF ADMINISTRATION	PERIOD OF TREATMENT	BLOOD PRESSURES*			
					Before constriction of renal arteries	Before treatment	During treatment	After treatment
1	Estrone	0.1 mgm./kgm.	i.m.	1 mo.	138-172/152	180-202/186	168-198/181	160-204/184
2	Testosterone	2.5 mgm./kgm.	i.m.	1 mo.	138-172/152	170-190/182	170-192/187	168-208/188
3	Liver extract	6 cc.	i.v., i.m.	1 mo.	140-152/146	149-170/166	157-190/172	152-192/164
4	Liver extract	12 cc.	i.v., i.m.	1 mo.	132-136/134	158-180/170	162-188/172	170-188/178
5	Pancreatic extract	0.3 gram	Per os	1 mo.	140-165/148	168-190/174	168-194/182	170-190/180
6	Garlic and parsley	2.0 grams	Per os	2 mo.	140-165/148	155-182/170	160-177/172	160-186/168
7	Renin	0.5 gram/kgm.	s.c.	2½ mo.	121-160/142	168-216/190	186-218/208	190-207/197
8	Renin	1.0 gram/kgm.	i.v.	1 mo.	111-140/127	160-184/172	180-195/188	153-186/173
9	Whole kidney	250 grams	Per os	1 mo.	138-172/152	172-204/192	172-212/196	180-206/198
10	Whole kidney	250 grams	Per os	1 mo.	112-118/115	160-182/172	164-182/176	162-194/180
11	Adrenal cortical extract	0.1 cc./kgm.	s.c.	1 mo.	111-140/127	154-175/168	156-182/166	160-184/172
12	Whole pituitary extract	1.5 grams/kgm.	i.m.	1 mo.	121-160/142	192-210/199	192-202/200	170-208/195
13	Pituitrin	1 cc./kgm.	i.m.	2 wk.	121-160/142	170-215/195	182-196/190	190-200/195
14	Pituitrin	1 cc./kgm.	i.m.	2 wk.	140-165/148	160-186/168	165-180/172	164-190/176
15†	Renin	0.5 gram/kgm.	s.c.	2½ mo.		112-130/122	122-150/134	122-127/125
16†	Renin	1.0 gram/kgm.	i.v.	1 mo.		134-148/143	145-172/154	140-150/145
17†	Adrenal cortical extract	0.1 cc./kgm.	s.c.	1 mo.		126-148/140	132-148/141	134-148/143

* Numerator figures denote extremes; denominator denotes average for period.

† Nonhypertensive dogs.

respectively in their blood pressures during the periods of injection. However, following the cessation of the injections the blood pressures fell to their preinjection hypertensive levels. Each of the normal dogs likewise showed a temporary average increase of approximately 10 mm. of Hg during the renin injection period.

b. Whole kidney. Two hypertensive dogs were given 250 grams of fresh hog kidney daily by mouth for one month without any significant effect on blood pressure.

c. Adrenal cortical extract.   An active adrenal cortical extract[6] was administered subcutaneously daily for one month to one hypertensive and one normal dog in a dosage of 0.1 cc. (7.5 Swingle dog units) per kilo. There was no change in the blood pressures.

d. Whole pituitary extract.   An aqueous extract of whole pituitary[7] was given daily intramuscularly for one month in a dosage of 1.5 grams of fresh gland equivalent per kilo to one hypertensive dog without any significant change in blood pressure.

e. Pituitrin.   A standard solution of posterior pituitary[7] in a daily dosage of 10 U.S.P. units per kilo intramuscularly to three hypertensive dogs for two weeks did not significantly alter the blood pressures of the animals.

The results for this second group of extracts are summarized in table 1.

Infections.   As previously reported (5) the appearance of distemper in four hypertensive dogs produced a prompt drop in the blood pressures to normal or subnormal levels.   When recovery occurred, the hypertension returned.   On the other hand an extensive mixed streptococcus and staphylococcus cellulitis in four hypertensive dogs did not lower their blood pressures.

DISCUSSION.   Estrogens and testosterone have both been reported to be of value in reducing the blood pressure in essential hypertension (6, 7). However, the best clinical evidence indicates that these hormones have no significant effect on the blood pressure in essential hypertension (8, 9) except in patients with complicating climacteric symptoms (10).   From the above results, it is apparent that estrone and testosterone in dosages 7 to 10 times those employed in the treatment of essential hypertension had no significant effect on the blood pressure of experimental renal hypertension in dogs.   The results with these two hormones in this form of experimental hypertension therefore agree substantially with those obtained by carefully controlled observations in essential hypertension.

As already stated, therapeutic claims for an antihypertensive effect in essential hypertension have been made for extracts of liver (11), pancreas (12), and garlic and parsley (13).   However, properly controlled evidence has shown that these and similar agents have no significant effect on the blood pressure of this condition other than that obtained with a properly administered placebo (3, 9).   There was likewise no significant effect from these extracts on the blood pressures of our renal hypertensive dogs even though on a weight basis the dosages administered were 7 to 10 times those recommended for the human.   The negative results with these extracts and the two sex hormones therefore offer a further paral-

lelism between experimental renal ischemic hypertension and essential hypertension.

There is suggestive indirect evidence that renin is the effective pressor agent released from the ischemic kidney in experimental renal hypertension and essential hypertension (2). The slight but significant temporary increase in blood pressure produced by repeated injections of dog renin as reported above constitutes further indirect evidence for a pathogenetic rôle for renin in experimental renal hypertension.

The known neutralizing effect of normal kidney on the pressor action of ischemic kidney in the intact animal (2) prompted our observation on the oral administration of fresh hog kidney. The negative results obtained may be due to inadequate dosage, ineffectiveness by mouth, or other factors. Very recently the successful extraction of an antihypertensive substance from normal kidney has been reported (14, 15).

The rôle of the adrenal cortex in experimental renal hypertension is still unsettled. Certain investigators have reported that the hypertension is maintained in completely adrenalectomized animals only if minimal amounts of the cortical hormone are supplied (2). Another group, however, found that hypertension can be produced and maintained in adrenalectomized dogs without supportive treatment during the survival period (16). The failure in our hands of a potent adrenal cortical extract to influence the blood pressure in experimental renal hypertension speaks against any direct effect of the adrenal cortex in the pathogenesis of the condition.

The results reported for hypophysectomy in experimental renal hypertension (17) indicate that the pituitary is not directly involved in the pathogenesis of the condition. Our failure to influence the blood pressure of renal hypertensive dogs with large doses of whole pituitary extract and of pituitrin is in keeping with this conclusion and contrasts with the pressor effect observed with dog renin.

The above findings, therefore, point to a possible rôle for renin in the pathogenesis of experimental renal hypertension in the dog and argue against such a rôle for the adrenal cortex and the pituitary. For essential hypertension the available evidence leads to similar conclusions (3).

CONCLUSIONS

1. Estrone, testosterone, and extracts of liver, pancreas, and garlic and parsley had no significant effect on the blood pressures of renal ischemic hypertensive dogs.

2. Dog renin temporarily increased the blood pressures of renal hypertensive and normal dogs slightly but significantly.

3. Fresh hog kidney, adrenal cortical extract, whole pituitary extract, and pituitrin were without effect on the blood pressure in experimental renal hypertension in dogs.

4. Distemper lowered the blood pressures of renal hypertensive dogs but extensive cellulitis was without effect.

5. The growing evidence of similarity between experimental renal ischemic hypertension in the dog and essential hypertension in man is further substantiated.

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THE MECHANISM OF THE ACTION OF DICARBOXYLIC ACIDS IN BLOOD COAGULATION

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The use of sodium citrate as an anticoagulant in blood transfusion led immediately to the study of its effect on the recipient. Weil (1) in 1915 records a 50 per cent reduction in the coagulation time in hemorrhagic conditions following administration of 5 grams of sodium citrate. Neuhof and Hirshfeld (2) attributed the effect of sodium citrate to platelet destruction. In 1934–35, De Souza and Hocking (3) studied the action of sodium citrate in producing a hypercoagulability of blood. They state that the intramuscular injection of sodium citrate rapidly makes the blood more coagulable, and increases the alkali reserve.

Noda (4) reported the action of gallic acid in decreasing coagulation time. A further report in 1939 by Noda (5) extends his original work to include dicarboxylic acids, aromatic acids of various types, and simple fatty acids. He states that the carboxyl is the essential group, and that its effect is increased by the presence of a hydroxyl group.

Steinberg and Brown (6) have further extended this work. They report the efficacy of malonic, maleic, fumaric, d,l-tartaric, glycollic, phthalic, mandelic, citric, sebacic, glutaric, adipic, pyruvic, azelaic, succinic acids, and ethyl and methyl oxalate.

Milliken (7) reports on the use of coagulant preparation containing dicarboxylic acid in transurethral prostatic resection. Davies (8) used intravenously administered oxalic acid in the control of hemorrhage in veterinary surgery.

Sterner and Medes (9) found that the *in vitro* addition of cysteine, taurine and taurocholic acid to whole blood delayed coagulation. The action of cysteine in inhibiting coagulation is on prothrombin, preventing its activation to thrombin. Putnam and Hoefer (10) also state that cysteine acts as anti-prothrombin.

Dicarboxylic acids in vitamin K deficiency. One of the first questions brought to mind by the foregoing observations is the possible effect of dicarboxylic acids in controlling the hemorrhages resulting from vitamin K deficiency. In investigating this possibility, we have employed chicks and used the technique of Ansbacher (11), using the ether-extracted fish

meal diet.   The changes in clotting times were determined by puncture of a wing vein with a fine needle; the blood was allowed to flow into a micro-test tube and its clotting time determined.   The acids in physiological saline were given intravenously, intraperitoneally, hypodermically and intramuscularly.   The clotting times were re-determined in six hours.   Table 1 summarizes the results.

TABLE 1

Anti-hemorrhagic effect of malonic acid in vitamin K deficiency

(Clotting times listed as average value for the group)

CHICKS	DOSE	ADMINISTRATION ROUTE	CLOTTING TIME BEFORE	CLOTTING TIME 6 HOURS
	mgm.			
6	0.25	Intravenous	>8 hrs.	18 min.
6	0.50	Intravenous	>8 hrs.	20 min.
12	0.25	Intramuscular	>2 hrs.	20 min.
6	0.50	Intramuscular	>2 hrs.	11 min.
3	0.12	Intraperitoneal	>1 hr.	14 min.
6	0.25	Intraperitoneal	>2 hrs.	12 min.
6	0.50	Intraperitoneal	>2 hrs.	11 min.
3	0.25	Hypodermic	>1 hr.	25 min.
6	0.5 cc. saline	Intravenous	>2 hrs.	>2 hrs.
3	0.5 cc. saline at pH 4	Intravenous	22 min.	>1 hr.
3	0.5 cc. saline at pH 4	Intramuscular	>2 hrs.	>2 hrs.
3	0.5 cc. saline at pH 4	Intraperitoneal	>2 hrs.	>2 hrs.

It would seem that the coagulation time of vitamin K deficient birds is reduced to nearly normal values by dicarboxylic acid therapy.   As the maximum effect of dicarboxylic acids in the normal animal occurs from $\frac{1}{2}$ to 1 hour after the injection, a redetermination of clotting times was made at varying intervals in an attempt to ascertain the time of maximum effect.   Our results in a series of 24 chicks over time intervals from 30 minutes to 7 hours showed no consistency.   The maximum effect might be reached in 30 minutes or in 7 hours.

Prothrombin times were determined by the method of Almquist and Klose (12).   As it is necessary to sacrifice the chick in order to determine these values, we used two series.   One series of untreated vitamin K deficient chicks showed prothrombin times which average 31 minutes for the group of 14.   Prothrombin times were taken on the vitamin K deficient treated chicks six hours after the administration of 1 mgm. of malonic acid.   The average value for the prothrombin times on this series of 9 chicks was 9 minutes.   Normal chick prothrombin time was determined to be 3 minutes.   These values demonstrate a pseudo increase in prothrombin levels.

In a smaller series of chicks, oxalic acid was tested for anti-hemorrhagic action in vitamin K deficiency with essentially the same results.

Studies of effect of dicarboxylic acids on coagulation of blood in normal animals. In the course of our investigations on the effect of various substances on coagulation time, we have used rabbits as experimental animals. A sample of blood was taken by cardiac puncture and immediately thereafter the substance to be tested was injected into an ear

TABLE 2

Effect of dicarboxylic acids on coagulation time of blood in normal animals

ACID	DOSAGE	COAGULATION TIME REDUCED TO	COAGULATION TIME PROLONGED
	mgm. per kgm.	*per cent*	*per cent*
Oxalic.....................	0.5–30	10–50	
Oxalic.....................	100		100–300
Malonic....................	3	20–60	
Succinic...................	3	25–60	
Glutaric...................	3	30–70	
Fumaric...................	3	No effect	
Maleic.....................	3	No effect	
Ascorbic	>10	75–85	
Gluconic...................	10	No effect	
Mucic......................	10	No effect	
Sodium acetate............	<10	No effect	
Sodium propionate........	<10	No effect	
Aspartic...................	0.5		100–300
Glutamic..................	0.5		25–50
Cysteine...................	1.0		90–150
Gallic......................	<5	No effect	
Salicylic...................	<5	No effect	
Benzoic....................	<5	No effect	
Phthalic...................	5	80–90	
Malic......................	>100	50–90	
Citric......................	>100	50–90	
Tartaric...................	>100	50–90	
Sulfuric....................	0.5 cc. of 0.03 N		50–200
Hydrochloric..............	0.5 cc. of 0.03 N		50–200

vein. After a half-hour interval, a second cardiac puncture was made and the coagulation time redetermined. Coagulation times were determined by collecting blood in a small test tube and determining the period elapsing from the moment the blood is shed to the time of congealing, as indicated by the inversion of the tube.

In our study of the mechanism of the action of dicarboxylic acids, it was necessary to observe the effect of a variety of acids on clotting time. The results of these observations are summarized in table 2.

The members of the oxalic acid series have a definite and powerful action in decreasing coagulation times. The activity decreases as the number of carbon atoms increases. This observation is entirely in conformity with that of Noda (5). Malonic acid, because of its greater solubility and lesser toxicity, seems the member of this group to be preferred in therapeutics. Oxalic, malonic, succinic and glutaric acids were tested as representative of this group. Rabbits of 5 lb. weight are used, and in these animals dosages of 1 mgm. of oxalic or malonic acid are effective in reducing the coagulation times to 10 per cent of their original values. Values of from 30 to 300 mgm. per kilogram are given as the fatal dose of oxalic acid, and as the therapeutic dose in reducing coagulation times is less than 1 mgm. per kilogram, it seems to be a safe compound to use. Toxic doses of oxalic acid (100 mgm. per kilo) produce the manifestation of a low calcium tetany and a marked prolongation in clotting time. We noted increases of 100 to 300 per cent in clotting times on blood taken when the animals were in a tetanic seizure. The exact concentration producing a transition from decreased to increased coagulation times is between 40 and 60 mgm.

Results with sodium fluoride. If the oxalate mechanism is primarily one involving calcium and its relationship to clotting, it would seem that the alkali salts of fluorine should have a similar action because the formation of the insoluble calcium fluoride should interfere with the clotting of blood in the same manner as does the formation of the insoluble calcium oxalate. Using the standard 5 lb. rabbits, we gave 0.1 mgm. to 5 mgm. intravenously with no effect on coagulation times. Doses of 10 to 60 mgm. decreased the coagulation time to as low as 30 per cent of the original value. Dosages of 200 mgm. and higher prolong the clotting times. A 200 mgm. dose is a toxic dose, and blood samples taken during the low calcium tetanic seizures show greatly prolonged clotting times, at times in excess of one hour. The entire picture is similar to that of the oxalate mechanism.

Results of in vitro experimentation. The experiments with fluoride and toxic doses of oxalate lead us to assume a calcium mechanism. *In vitro* experiments show exactly the same results as *in vivo* experiments. The addition of 0.2 cc. of 0.001 molar sodium oxalate to 1.8 cc. of blood reduces coagulation times by 5 to 60 per cent; while 0.2 cc. of 0.1 molar sodium oxalate prevents blood from clotting. The addition of 0.2 cc. of a saturated $CaCl_2$ solution to 1.8 cc. of blood prevents clotting or prolongs it to in excess of 30 minutes. The addition of traces of calcium ion to blood slightly hastens clotting.

Action of dicarboxylic acids on heparinized blood. If it is true that dicarboxylic acids act on the first phase of coagulation, it follows that dicarboxylic acids should have no effect on coagulation time in heparinized

animals.   Heparin is known to have an anti-thrombase action which in-
hibits the second phase of blood coagulation.   Rabbits weighing 2.5 kgm.
were given 5 mgm. of heparin intravenously after their normal clotting
times had been determined.   The clotting time was redetermined five
minutes after the heparin was injected.   Simultaneously, oxalic acid (10
mgm.) was injected intravenously, and 15 minutes later the clotting time
was redetermined.   The results are summarized in table 3.

It is apparent that the dicarboxylic acids had no significant effect on
the clotting time of heparinized blood.   This fact points to the first phase
of coagulation as the one affected by dicarboxylic acids.

DISCUSSION.   All of these facts taken in conjunction point to the
existence of a calcium prothrombin or similar calcium combination.   With

TABLE 3

The effect of oxalic acid on the coagulation time of heparinized animals

RABBIT, 2.5 KGM.	CLOTTING TIME BEFORE HEPARIN	CLOTTING TIME 5 MIN. AFTER INJEC-TION OF 5 MGM. HEPARIN	CLOTTING TIME 15 MIN. AFTER INJEC-TION OF 10 MGM. OXALIC	PER CENT CHANGE IN CLOTTING TIME EF-FECTED BY THE AD-MINISTRATION OF OXALIC ACID
				per cent
1	5' 30"	60'	25'	41.5
2	3' 25"	85'	35'	41.1
3	3' 23"	40'	20'	50.0
4	3' 11"	26'	11'	42.0
Heparinized but not treated with oxalic acid				.
5	4' 5"	85'	37'	43.5
6	3' 21"	67'	28'	41.8

this one assumption, based on the established existence of calcium pro-
teinate combinations, the entire picture becomes harmonious.

The addition of small amounts of oxalate to blood *in vitro* and the
intravenous administration of oxalate reduce coagulation time, due to
the withdrawal by the oxalate of calcium ion from the blood.   Then a
simple application of apparent solubility product and mass action law
shows that the calcium prothrombin union is broken up, freeing the
prothrombin for its subsequent conversion to thrombin.   This prothrom-
bin-to-thrombin reaction is catalyzed or facilitated by the presence of
calcium ions, and if enough oxalate is added to remove the calcium ion,
greatly delayed clotting or no clotting is the result.   The dissociation
constant and apparent solubility product of the calcium prothrombin
combination are smaller and larger respectively than the corresponding
values for the calcium oxalate compound, which means that complete
dissociation of the calcium prothrombin combination would occur before

the oxalate removed the calcium ion from the blood sample. Thus, the addition of small amounts of oxalate increases the amount of prothrombin free and available for conversion to thrombin; but it does not remove the calcium ions, and hence clotting is hastened. Conversely, the addition of excess calcium by mass action effect forces all of the prothrombin into combination and leaves none in a free condition for conversion to thrombin. Hence, inhibition of the clotting of blood.

The following chart illustrates in diagrammatic form the reactions involved:

Outline of Reactions Involved in the Oxalate Mechanism

$$\text{Calcium Prothrombin} \rightleftharpoons Ca^{++} + \text{Prothrombin} + \text{Oxalate}^{-} \rightleftharpoons \text{Calcium Oxalate}$$

$$\downarrow Ca^{++}$$

$$\text{Thrombin} + \text{Fibrinogen}$$

$$\searrow$$

$$\text{Fibrin}$$
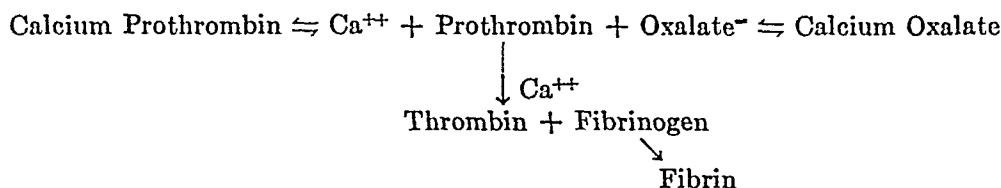
Cephalin combines with and removes the anti-thrombin, heparin.

1. Add calcium ion in excess:

Result: $\dfrac{(Ca^{++})\ (\text{Prothrombin})}{(\text{Ca Prothrombin})} > K$

Therefore calcium ion and prothrombin combine to form calcium prothrombin until

$\dfrac{(Ca^{++})\ (\text{Prothrombin})}{(\text{Ca Prothrombin})} = K$

Leaving a minimum of free prothrombin for conversion to thrombin, and clotting times are prolonged.

2. Add small amount of oxalate ion:

Result: $\dfrac{(Ca^{++})\ (\text{Oxalate}^{-})}{(\text{Ca Oxalate})} > K$

Therefore calcium and oxalate ions combine to form calcium oxalate with the consequent withdrawal of calcium ions causing

$\dfrac{(Ca^{++})\ (\text{Prothrombin})}{(\text{Ca Prothrombin})} < K$

Therefore calcium prothrombin dissociates into calcium and prothrombin, liberating prothrombin for conversion to thrombin and hastening coagulation.

3. Add large amount of oxalate:

Result: $\dfrac{(Ca^{++})\ (\text{Oxalate}^{-})}{(\text{Ca Oxalate})} > K$

Therefore calcium and oxalate ions combine to form calcium oxalate with the resultant removal of nearly all of the calcium ion. This results in complete dissociation of the calcium prothrombin combination, but the calcium ions concentration is too low to catalize the conversion of prothrombin to thrombin reaction.

Mathematically, it is possible to calculate the calcium ion concentrations if certain assumptions are made. The volume of blood in a 2.5 kgm. rabbit must be assumed to be 250 cc. The calcium ion concentration must be assumed to be 5 mgm. per 100 cc. Finally, the oxalate ion concentration must be assumed to be 4 mgm. per 100 cc. From these values the apparent solubility product is calculated to be $(4.8) (10^{-7})$. Using this value for the apparent solubility product of calcium oxalate, we find the calcium ion concentration of the rabbit's blood following the administration of 60 mgm. dose of sodium oxalate to be $(2) (10^{-4})$ moles per liter. This is a value of calcium ion concentration which results in an inhibition of the clotting processes. The same value following a 40 mgm. dose of sodium oxalate is $(4) (10^{-4})$. Thus at an approximate calcium ion concentration of $(2) (10^{-4})$ moles per liter, coagulation is inhibited, while at a calcium ion concentration of $(4) (10^{-4})$ moles per liter coagulation is hastened.

It is impossible to calculate similar values for any other acid ion used as no values are known from which the apparent solubility products can be calculated. It is to be emphasized that we apply the law of mass action only to the reactions designated and not to the entire process of clotting. Moreover, we realize that we are measuring speed of a reaction and therefore cannot directly apply the law of mass action to the entire process; but inasmuch as calcium ion concentration and free prothrombin concentration are known to control the speed of the reaction, we feel justified in applying the law of mass action indirectly to the entire process, as we apply it directly to the reactions listed.

The study of the action of dicarboxylic acids in normal animals leads us to suggest a similar explanation for the effects observed in hypo-prothrombinemia due to a lack of the anti-hemorrhagic vitamin. A calcium prothrombin combination exists in the blood. For its conversion to thrombin, the prothrombin must be liberated from the combination. This is the action of the dicarboxylic acid. The mobilization of the prothrombin results in the decreased clotting times observed by us.

SUMMARY

Dicarboxylic acids reduce coagulation time in vitamin K deficient chicks but do not alter coagulation time in heparinized animals. Fluorides reduce coagulation times in normal animals. Evidence is presented indicating that the action of fluorides and dicarboxylic acids is one determined by the law of mass action involving directly calcium ion concentrations and indirectly prothrombin concentration. A calcium prothrombinate is hypothesized.

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THE EFFECTS OF PHLORHIZIN ON RENAL PLASMA FLOW, ON GLOMERULAR FILTRATION AND ON THE TUBULAR EXCRETION OF DIODRAST IN THE DOG[1]

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The action of phlorhizin in suppressing the tubular reabsorption of glucose is well known; knowledge of its action on transfers from blood into tubular lumen is less complete. Under phlorhizin the normal excess of exogenous creatinine over inulin clearance in man (1), the apes (2), the chicken (3), and the teleost (4) and clasmobranch (5) fishes is abolished or reduced; this has been interpreted as meaning that it abolishes or reduces a preëxisting tubular excretion of creatinine. Similar evidence has been cited (6) for a depression of the tubular excretion of creatine by phlorhizin in the marine teleost, *E. morio*, although the tubular excretory mechanism for creatine in the dogfish is reported as not being affected by phlorhizin except where renal circulation is severely depressed (7). Phlorhizin has no effect on the tubular excretion of phenol red in the dog (8), except for transitory depression which appears to be due to circulatory changes. In the chicken phlorhizin depresses the tubular as well as the glomerular excretion of phenol red, "but whether this is a result of a reduction of blood flow to the kidney or whether the tubular mechanism by which phenol red is excreted is specifically affected, cannot be determined from the present data" (9). The question of a direct action of phlorhizin on tubular excretory mechanisms appears to be unsettled except in the case of creatinine, where evidence for such action is strongest.

Difficulties of interpretation have been increased by the circumstance that in most of the above cited work doses of phlorhizin designed to block completely the reabsorption of glucose have been used. Intravenous administration was usually employed, alone or supplemented by subcutaneous administration; circulatory disturbances are often produced, sometimes with nausea and vomiting in mammals. Such disturbances are negligible in dogs if a dose of not more than 0.2 gram per kilo is given subcutaneously and 1.5 hours allowed to elapse before clearance observations are begun. Such a procedure may or may not completely phlor-

hizinize the dog, where identity of glucose with inulin or creatinine clearance is the criterion of completeness. In any event the glucose clearance will be 80 per cent or more of the inulin. This effect is adequate for our purpose, which is to see whether a dose of phlorhizin adequate to bring on marked if not complete suppression of glucose reabsorption, with little or no circulatory effect, will affect tubular excretion of diodrast (D). As a further check on what is happening to the renal circulation, beyond that afforded by the behavior of the absolute values of inulin and D clearances, renal plasma flow (RPF) was determined in two experiments on two renal explant dogs by following the extractions of inulin and D. A depression of the tubular excretion of D by phlorhizin was found, over and above any effect due to circulatory changes.

METHODS. Inulin, glucose and D plasma clearances were carried out either at constantly maintained plasma levels throughout the experiment, or with falling plasma levels of inulin and D following two urine periods within which a constant and high level of D was maintained. Four phlorhizin experiments with 18 urine periods have been carried out on 4 dogs; many more control or nonphlorhizin periods were obtained on these dogs. In 3 experiments 0.2 gram of phlorhizin per kilo, as a 10 per cent solution in 2 to 2.5 per cent sodium bicarbonate solution, was given subcutaneously 1.5 hours before the first blood collection; in one experiment 0.1 gram per kilo was given intravenously over a 12 minute period, with the next blood collection 17 minutes after the end of the phlorhizin injection. Maximum rates of tubular excretion of D (Tm) have been followed, as well as clearances at lower plasma D levels. Trained, unanesthetized female dogs were used; inulin (10) and D (11) were given by intravenous infusion; a Shaffer-Somogyi copper reagent was used in glucose determinations. Urine inulin readings were corrected for glucose contribution to color; glucose effect on plasma inulin readings was compensated by using a blank filtrate (before inulin administration) for the zero setting in the colorimeter. This precludes the necessity of glucose removal by fermentation, since our plasma glucose levels remained very constant, with variations of less than 5 mgm. per cent throughout an experiment.

RESULTS. *Diodrast plasma clearance.* At all plasma D levels investigated the D plasma clearance under phlorhizin is considerably below the normal for the corresponding plasma D level. Figure 1 shows normal clearances on 4 dogs as solid circles and clearances under phlorhizin as open circles on the same dogs. The percentage reduction at low plasma D levels is somewhat greater than at high levels; this is explained by the following circumstances. The higher the plasma level above that at which self-depression of D clearance begins (about 13 mgm. I per 100 cc.), the greater is the percentage of D excretion contributed by the glomeruli.

At a plasma level of 30 mgm. I per 100 cc. the excretion of D in normal dogs is equally divided between glomeruli and tubules and as the level rises the glomerular contribution rises above 50 per cent. If phlorhizin depresses only the tubular contribution, it follows that its effect on total clearance will be less at high plasma levels, where the glomerular contribution becomes more and more important. One would thus predict from an inspection of figure 1 that glomerular filtration rate (inulin clearance) is less affected by phlorhizin in these experiments than is tubular excretion of D, and this is found to be true.
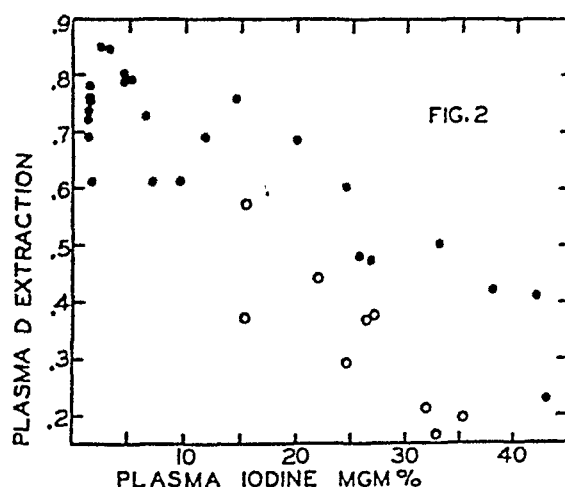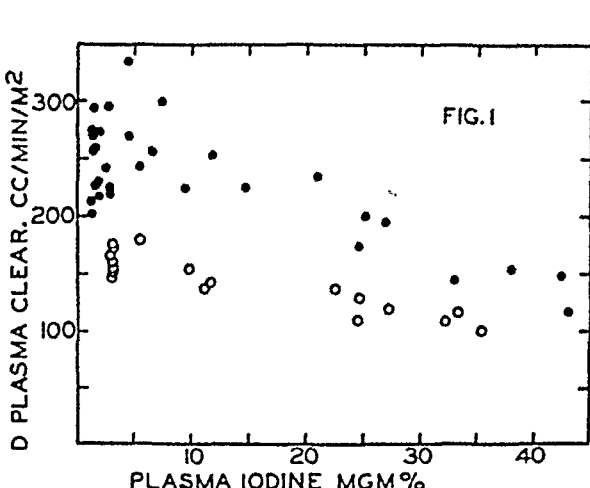
Fig. 1. Comparison of normal D plasma clearances (solid circles) with D plasma clearances under phlorhizin (open circles) at various plasma I levels, I being a measure of D.

Fig. 2. Comparison of plasma D extractions in normal (solid circles) and phlorhizin (open circles) periods at various plasma I levels.

Extraction of diodrast. As would be predicted from the clearance effects, the plasma extraction, $\dfrac{A - RV}{A}$, of D is lowered by phlorhizin. Figure 2 shows plasma D extractions under phlorhizin as open circles, as compared with extractions without phlorhizin. Renal vein bloods were not obtained in the 2 experiments (7 periods) with phlorhizin at plasma levels of about 3 mgm. I per 100 cc. seen in figure 1.

Renal plasma flow. The preceding 2 sections indicate that the lowered D plasma clearance seen under phlorhizin is due to tubular depression but do not afford unqualified proof of this. This proof can be given by showing that the effects cannot be referred to circulatory changes. If it can be shown that RPF is not lowered and that glomerular filtration rate is not greatly lowered by phlorhizin in these experiments, it follows that the depression of clearance must be due to a specific depression of tubular

excretion by the drug. Renal plasma flows were obtained on 2 dogs, as inulin plasma clearance/plasma inulin extraction and as D blood clearance $\times$ V_p/blood D extraction (12); the 2 methods agree closely and their average is given here. One dog (K1) showed an average normal RPF of 285 cc./minute/M² while the phlorhizin periods averaged 307; the other dog (K3) averaged 396 normal and 408 with phlorhizin.

Glomerular filtration rate. The inulin clearance in the 3 dogs where 0.2 gram phlorhizin subcutaneously was given 1.5 hours before the beginning of the clearance observations averaged 94 cc./minute/M² in the normal periods and 67 in the phlorhizin periods. The dog (K7) which was given 0.1 gram per kilo intravenously 17 minutes before the next period started averaged 71 cc./minute/M² in the normal and 60 in the phlorhizin periods. It thus appears that some fall in glomerular filtration results from phlorhizin as given in these experiments. This fall, however, is quite inadequate to account for the total fall in D plasma clearance, as will be pointed out later. Since it is not accompanied by any fall in RPF, it must be explained by a slight constriction of the afferent vessels, presumably accompanied by a corresponding dilatation distally.

Tubular clearance of diodrast. One can get an expression for tubular clearance of D by dividing tubular output in mgm./minute/M² by plasma level; tubular output is total output minus glomerular output, the latter being calculated from filtration rates and filterable fraction of D (13). If the fall in total (glomerular plus tubular) plasma clearance of D under phlorhizin were due solely to the fall in glomerular filtration rate, the tubular clearance would remain unchanged; actually it is considerably reduced. Taking only the periods where plasma I level is below 13 mgm. per 100 cc., i.e., the periods in which there is no self-depression of D clearance, the 4 dogs show an average tubular D clearance of 173 cc./minute/M² in the normal and 110 in the phlorhizin periods. The average total plasma clearance of D drops from the normal of 267 cc./minute/M² in these experiments to 177 under phlorhizin; of this drop of 90, the fall in glomerular output accounts for only 27. Depression of tubular output by phlorhizin thus accounts for 70 per cent of the drop.

Even though it is shown that the tubular excretion of D is lowered by phlorhizin it is still not proved that any specific disturbance of the excretory mechanism is being produced until it is shown that the fall cannot be explained merely on the basis that less D is being brought to the tubules. The best expression of the tubules' efficiency in excreting D is what may be termed the "tubular extraction" of D, which can be expressed as tubular D plasma clearance/RPF. It designates the fraction of RPF which is cleared of D *by tubular activity.* A fall in this value indicates a fall in the inherent ability of the tubules to excrete D and its value will

not be affected by changes in RPF unless the latter falls so low as to damage the tubules by ischemia, which is not the case in these experiments. This fraction has averaged 0.55 in the normal periods and 0.31 in the phlorhizin periods. It thus appears that the efficiency of the tubules in excreting D at plasma levels below 13 mgm. I per 100 cc. is lowered by phlorhizin in these experiments to about 56 per cent of the normal and that the depressant effect cannot be explained on the basis of a tubular ischemia.

Effect on maximum tubular excretory rate. Observations on the maximum rate of tubular excretion (Tm) of D as affected by phlorhizin have been made on 2 dogs. In these dogs D Tm was normally attained at a plasma level of 18 to 20 mgm. I per 100 cc. and averaged 24.3 mgm. I/minute/M^2; under phlorhizin the plasma level necessary to bring on D Tm was the same as in the normal periods (somewhere between 16 and 20 mgm. I per 100 cc.) but the value of D Tm averaged 15.5 mgm./minute/M^2. The maximum ability of the tubules to excrete D under phlorhizin is thus 64 per cent of that without phlorhizin; here also the effect is not due to any fall in RPF. It thus appears that the maximal tubular excretory power for D is depressed by phlorhizin in these experiments to about the same extent as is their efficiency in extracting D at low plasma levels, i.e., to about 60 per cent of the normal; this identity of effects at high and low plasma levels is of some theoretical interest.

Glucose clearance. The tubular reabsorption of glucose was usually not completely abolished by phlorhizin as given here; the ratio glucose/inulin plasma clearance averaged 0.85. With phlorhizin doses and time relations adequate to insure a unity glucose/inulin clearance ratio an even greater depression of tubular excretion of D would probably be obtained. It was considered more desirable, however, to restrict the phlorhizin dose so as to minimize circulatory changes even though glucose reabsorption was not completely suppressed, since our arguments do not demand such completeness.

SUMMARY

A dose of 0.2 gram phlorhizin per kilo subcutaneously given 1.5 hours before beginning observations lowers the plasma clearance and renal extraction of D considerably more than can be accounted for by the slight lowering of glomerular filtration rate which results. Renal plasma flow is unchanged. The ability of the tubules to excrete D is lowered to about 60 per cent of the normal at both high and low plasma D levels. The work was done on unanesthetized dogs.

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THE GREATER RESISTANCE OF VERY YOUNG ANIMALS TO ARREST OF THE BRAIN CIRCULATION[1],[2]

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Previous investigations have revealed that the young animal is more resistant to asphyxia than is the adult (1, 2). Recently, it has been demonstrated that the brain itself is more resistant in the young animal, since young dogs were found to be less susceptible than adults to complete arrest of the brain circulation (3). Careful studies of the changes in vascularity in various portions of the rat brain from birth to maturity (4, 5, 6) have revealed a rapid and progressive increase in vascularity during the developmental period. It was therefore deemed advisable to investigate quantitatively the course of the change in resistance to arrest of the brain circulation during the period of rapid growth.

The basis for such a quantitative investigation is the remarkable constancy of the effects produced by a given period of complete cephalic stasis in adult dogs (7, 8). Thus, all normal adult animals studied had recovered completely several weeks after arrest of cephalic blood flow for a period of six minutes or less. On the other hand, severe permanent brain damage was evident in every adult dog subjected to eight minutes of arrest of the brain circulation. The symptoms and the course of recovery of adult dogs subjected to the same period of arrest of blood flow were practically identical regardless of differences in size, age, sex or breed of the experimental animals.

METHOD. The method employed was essentially the same as had been used previously in the investigation of adult dogs (7, 8). A preliminary aseptic operation, involving removal of the spine and laminae of the second cervical vertebra, was performed under ether or pentobarbital a day or two before the experiment.

A day or two after operation, following insertion of a metal tracheal tube through the glottis and administration of atropine, a cervical pressure cuff

was inflated suddenly by means of compressed air to a pressure of 600 to 800 mm. of mercury. In this way, the blood flow to the brain was stopped suddenly and completely. Artificial respiration was given when the respiratory center began to fail, and was continued during and following the arrest of circulation until respiratory function was again established. The effects of arrest of the cephalic circulation have been studied in 42 dogs, ranging in age from 8 to 141 days. The animals varied in weight, sex and breed. Littermates were studied using different periods of brain stasis.

The argument that the high pressure over the spinal cord in the region of the laminectomy may cause damage to the spinal cord and thus complicate the results has been discussed elsewhere (8). It will be evident from the results to be presented that this is not a significant factor.

RESULTS. *A. Quantitative study of revival time of brain function.* The maximal duration of arrest of the cephalic circulation from which brain function can recover completely has been studied in dogs at different ages. This "revival time" (9) decreases progressively as the animal grows older. This study is illustrated graphically in figure 1. All points on or below the lower curve (absolute revival time) represent animals which recovered completely in a relatively short time following arrest of cephalic blood flow and lived a normal existence for months afterwards. This curve, then, represents the duration of cephalic stasis from which animals can undoubtedly recover completely. It is, however, questionable whether these figures represent the maximal period of cephalic stasis compatible with complete recovery. One needs only to recall that adult dogs, following a six-minute period of cephalic stasis, remain in coma for 24 to 36 hours and show neurological symptoms such as ataxia for several weeks, after which recovery is apparently complete (8). While the animals represented in figure 1 by open circles had neurological symptoms until they died, most of them only lived for 1½ to 10 days following arrest of the cephalic circulation. The symptoms which they showed were relatively mild in most instances. These animals were able to stand and walk, to lap milk, swallow and sometimes nurse on the day following the arrest of cephalic blood flow. Their symptoms consisted of ataxia, tremor and lack of spontaneous activity. Furthermore, many of these young animals showed improvement during the few days that they remained alive, and it is not excluded and even seems probable that recovery would have been complete had they survived for a longer period. The animals died either of secondary upper respiratory or gastrointestinal infection or of poor nutrition and dehydration. When one considers the difficulties involved in feeding and caring for these young nursing animals, such results are not at all surprising. It is necessary, therefore, to revise our estimates of revival time upward and the upper curve in figure 1 seems likely to be closer to the correct figures.

The change in resistance to arrest of the cephalic circulation with age

is thus well shown by the study of revival time. At every age, there appears to be a critical revival time beyond which complete recovery is impossible, and this is as true at 8 to 10 days of age as it is in the adult. While the adult has a revival time of 6 minutes, at 79 days the revival time is 10 minutes, at 48 days 12 minutes, at 24 days 14 or 15 minutes, at 10
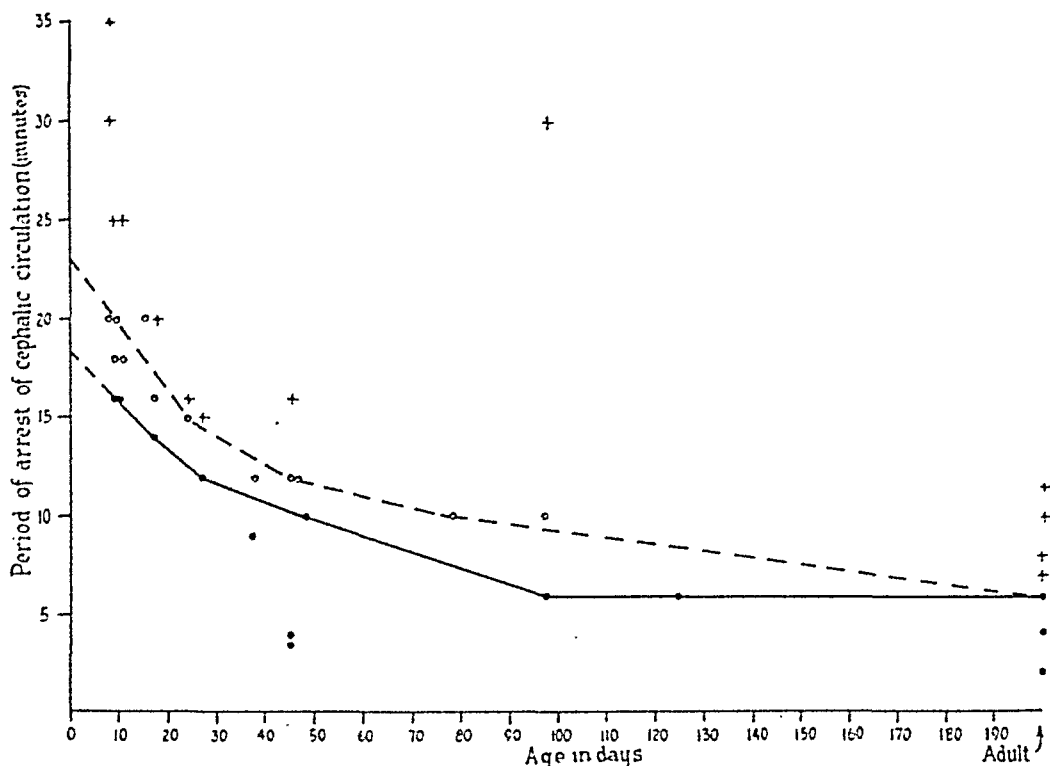
Fig. 1. Maximum period of cephalic stasis compatible with complete recovery of brain function at various ages (revival time). The solid circles represent animals which showed complete recovery of brain function and lived for months after cephalic stasis. The solid line indicates the absolute revival time. The open circles represent animals which had some symptoms of brain damage following cephalic stasis and remained alive for 1½ to 10 days. The dotted line indicates the possible or probable revival time. The crosses represent animals which showed evidence of severe brain damage and died as a rule within 24 hours. Further explanation will be found in the text.

days close to 20 minutes. At birth, continuing this trend, one might expect a revival time of approximately 23 minutes.

The decrease in resistance of the brain to stasis with increasing age of the developing animal was also brought out clearly by the study of littermates at different ages. Two littermates, A and B, males of the same size, weight and color, were subjected to arrest of the cephalic circulation, A at the age of 27 days and B at the age of 124 days. On the day following a 12-minute period of arrest of cephalic blood flow, A was awake and could

stand and walk but fell frequently as a result of ataxia. At 48 hours the animal was entirely normal except for a slight ataxia which soon disappeared. B at the age of 124 days was subjected to a period of cephalic stasis of six minutes, only half the period of stasis to which the littermate had been subjected some three months earlier. For the first 48 hours after arrest of blood flow of six minutes' duration, B was unconscious or had only very dim consciousness of his surroundings. He was unable to turn from his side to his abdomen, stand or walk, and lay on his side showing no spontaneous movements. It was not until the fifth day that he regained full consciousness, could turn from his side to his abdomen and sit up. He was still unable, at this stage, to stand or walk. At the end of a week, he was alert and curious and was able to stand and walk but fell very frequently due to the severe ataxia. The ataxic symptoms gradually improved, and disappeared a month after the cephalic stasis. Both A and B are still alive at this writing, $8\frac{1}{2}$ months and $5\frac{1}{2}$ months, respectively, after the arrest of blood flow, and neither animal can be distinguished from the normal. The effects of a 6-minute period of arrest of brain circulation were thus more severe at 124 days of age than a 12-minute period of circulatory arrest in a littermate 27 days of age.

A similar experiment was performed in which two littermates were subjected to arrest of cephalic circulation of ten minutes' duration at different ages. Dog 9, following 10 minutes of arrest of blood flow at the age of 48 days, was able to stand and walk ataxically after three hours. After six hours, he was alert, curious and responsive, came when he was called, fed himself and was quite normal except for a slight ataxia, and after 24 hours had recovered completely. On the other hand, dog 10, at the age of 77 days, had much more severe symptoms following 10 minutes of cephalic stasis. On the day after the arrest of circulation, the animal lay on his side in coma, motionless and unable to turn from the side to the abdomen. By the fourth day, he had recovered consciousness and could sit up and eat but was unable to stand or walk. On the fifth day he was more curious, active and responsive, and could stand and walk, although the very severe ataxia resulted in frequent falling. Thus, dog 10, although only a month older than his littermate, was severely affected by the same period of cephalic stasis from which the littermate had recovered in a few hours.

B. *Loss of function during complete arrest of the cephalic circulation.* The time during which various functions persist, despite complete cessation of blood flow in the brain, is an index of the resistance of the neural structures to anoxia which has been designated the "survival time" (9). Studies of the corneal reflex, respiration and spontaneous somatic movements in dogs at various ages show that the survival of reflexes of animals 8 to 11 days of age is very much longer than in adult dogs. The index of

resistance to arrest of cephalic blood flow (adult = 1), derived from the data on respiratory survival time, provides a rough estimate of the quantitative change in resistance with age. When the index of resistance of the respiratory center is plotted against age, one obtains a hyperbolic curve (fig. 2), indicating that the decline in resistance to anoxia is very rapid during the first post-natal month and slows down progressively in the second, third, and fourth months of life. An animal at four months of age is as susceptible to cephalic stasis as the adult dog. When the curve is extrapolated to zero age (birth),[3] the resistance of the respiratory center to arrest of its circulation appears to be approximately seventeen times that of the adult. The respiratory survival time during brain stasis is 20–30 seconds in the adult dog and five minutes in dogs 8 to 10 days of
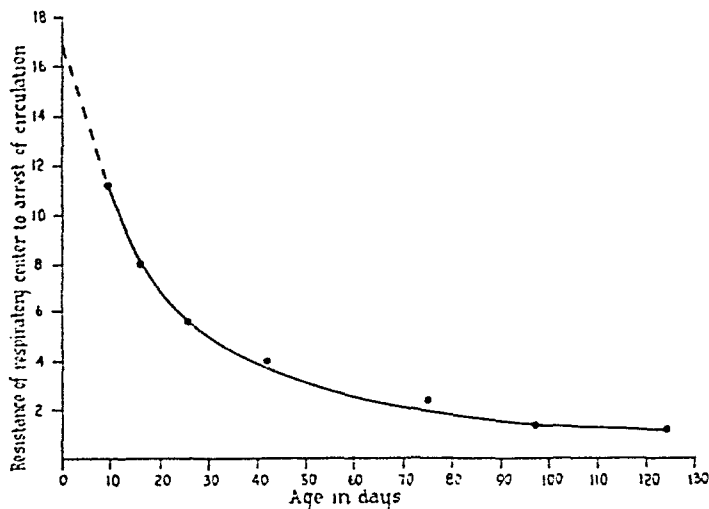
Fig. 2. Influence of age on the resistance of the respiratory center to arrest of circulation. The resistance of the respiratory center represents the index of relative resistance based on survival time, with adult = 1.

age. Respiratory efforts persist for 27 minutes during asphyxia in dogs 1 day old, while they cease in 2 to 4 minutes (10) in asphyxia in the adult.

During arrest of cephalic blood flow in the adult dog, after cessation of respiratory reflexes and of spontaneous somatic movements, a state of profound spinal shock becomes evident (8). This is readily made apparent by disappearance of the flexion reflex to noxious stimulation of the foot. The spinal shock often persists for some time after arrest of blood flow and spinal reflex irritability is only gradually restored. In young dogs, on the other hand, there was no evidence of spinal shock during complete arrest of blood flow in the brain. In eleven animals, 8 to 11 days of age, the flexion reflex could be elicited readily throughout the period of cephalic

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[3] The validity of such extrapolation may be open to question.

stasis, even though the stasis was continued for as long as 35 minutes. The flexion reflex was also present throughout the period of arrest of cephalic circulation in dogs 17, 24, and 27 days of age. Evidence of mild spinal shock was found in experiments on dogs 45 days of age. In a dog 97 days of age subjected to complete arrest of brain circulation for a period of 6 minutes, the flexion reflex could no longer be evoked after 2 minutes, 50 seconds of stasis, and the reflex did not reappear until one minute after restoration of blood flow. At four months of age, spinal shock appeared during cephalic stasis and was only gradually dissipated afterwards. At first, after restoration of blood flow in the brain, only the flexion reflex was elicited and the crossed extension reflex did not reappear until several minutes later.

C. *Immediate return of function after restoration of cephalic blood flow.* After the blood was allowed once more to flow through the brain, this organ gradually recovered its functions. These functions were restored in the order opposite to their disappearance during cephalic stasis. The first sign of recovery was movement of the tail or movements of the skin followed by movements of the limbs. Soon after this, the first inspiration occurred, a strong inspiratory movement accompanied by opening of the mouth, repeated irregularly and infrequently. Some time after the beginning of respiratory efforts, the corneal reflex was restored and after another short interval, respiration became normal in rhythm and frequency.

The speed of recovery of the lower centers involved in these functions depends on two factors: the duration of arrest of the brain circulation, and the age of the animal. The quantitative results on "recovery time" of respiration (appearance of the first inspiration) and of the corneal reflex in dogs at various ages is illustrated in figure 3. Most of the points represent the average recovery time of a number of animals. Restoration of function of the corneal reflex is greatly delayed compared to the respiratory center, being on the average about four times that of respiration. For any period of cephalic stasis, the younger the animal, the shorter the recovery time and, with increasing age, the recovery time of the young animal approaches that of the adult dog. In general, regardless of age or the duration of cephalic stasis, when the respiration is revived within $2\frac{1}{2}$ minutes and the corneal reflex within 12 minutes, later recovery of brain function is apparently complete. On the other hand, longer recovery times beyond these critical values result in more or less severe permanent damage of the brain. (One must keep in mind that these are average figures and must allow for individual variations.) The average critical figures for recovery time are somewhat lower for the adult dog, being two minutes for respiration and less than eight minutes for the corneal reflex.

D. *Asphyxia in the neonatal period.* Anoxia was produced in 11 dogs and 6 rats in the neonatal period by causing the animal to rebreathe a

small volume of pure nitrogen. In the dogs, a small metal cone was employed, the open end of which was covered with a rubber membrane containing an opening just large enough to admit the head. This cone was filled with nitrogen by blowing a stream of the gas through it for a minute or two, following which the cone was placed in position. The stretched rubber membrane fitted around the neck and was air-tight. The rats were placed in a short wide test tube full of nitrogen which was stoppered.

The results are presented in table 1. It is interesting to compare these results with the effects of asphyxia in the adult dog. According to
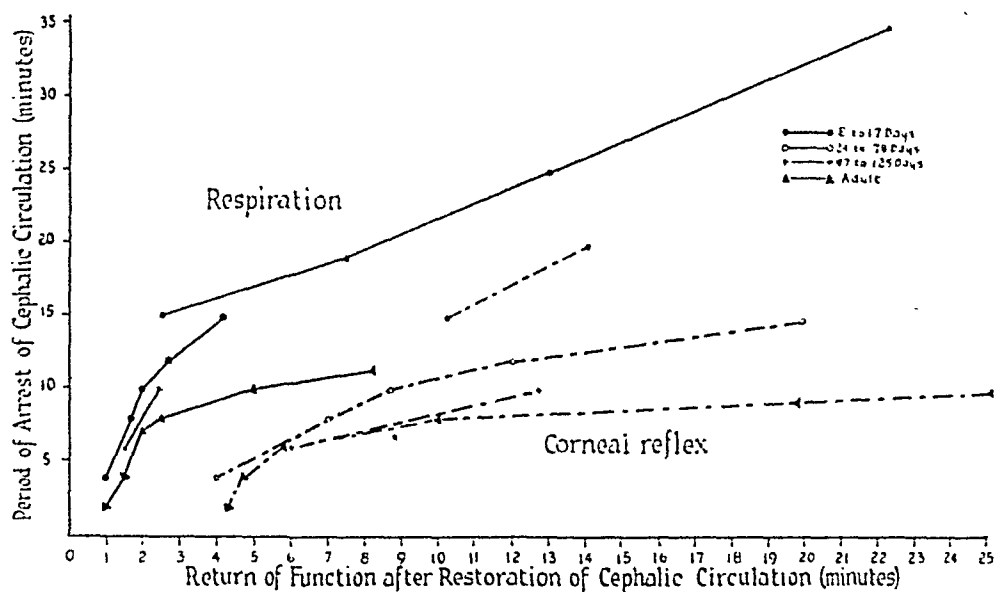
Fig. 3. Recovery time of respiration and the corneal reflex in dogs at various ages. The solid lines represent recovery time of respiration (first gasp); the broken lines represent recovery time of the corneal reflex. The symbols for the various age groups are indicated in the figure. Most of the points represent the average recovery time of a number of animals. The data for the corneal reflex in the youngest age group were very meager because most of these animals did not yet have the eyes open.

Lougheed, Janes and Hall (10), adult dogs never recover after seven minutes of acute asphyxia, rarely recover after four minutes, and in some cases do not recover after only two minutes of asphyxia.· In all of the dogs one day of age which died (1, 4 and 5), inspiratory movements returned during the first minute of resuscitation but persisted only 4 to 5 minutes and then stopped. Soon after cessation of respiration, the flexion reflex disappeared. It was then found impossible to resuscitate the animal, despite prolonged and efficient artificial respiration. In the two other experiments which terminated fatally, no respiratory effort was made following renewal of the oxygen supply.

All of the young dogs and rats which survived the severe acute asphyxia recovered function very rapidly. The animal which recovered from anoxia of 25 minutes' duration sat up and moved 11 minutes later and became active and began to nurse 17 minutes after the end of anoxia. After 20 minutes of anoxia, the newborn animal, though unable to turn from the

TABLE 1

Acute anoxia in the neonatal period

DOG	AGE	Duration of anoxia	Respiration stops	Movements cease	Flexion reflex	First inspiration	Flexion reflex	Result
			LOSS OF FUNCTION			RETURN OF FUNCTION		
	days	*min.*	*min.*	*min.*				
1	1	19	18	18	18 min. 25 sec., weak; 18 min. 40 sec., gone	50 sec.	1 min., good	Respiration stopped at 4 min., died
2	1	20	16	8	13 min., weak; 16 min., gone (pinch foot causes gasping)	4 min.	Returns	Lived
3	1	25	23	20	Good throughout	20 sec.	Never lost	Lived
4	1	35	34	17	Good throughout, weaker at end	45 sec.	30 sec., good; 2 min. 30 sec., gone; 3 min., weak	Respiration stopped at 5 min. 45 sec., died
5	1	41	40	30	40 min. 30 sec., weak	1 min.	30 sec., weak	Respiration stopped at 4 min. 40 sec., died
6	2	15	14			None		Died
7	2	17½	14			None		Died
8	3	16	14			4 min.		Lived
9	3	17	16			3 min.		Lived
10	9	7	Continues			Never lost		Lived
11	9	17	16	6		3 min.		Lived
RAT								
1	1½	30	Continues	Continues		Never lost		Lived
2	1½	40	Continues	Stopped		Never lost		Lived
3	1½	44	Continues	Stopped		Never lost		Lived
4	1½	45	Continues	Continues		Never lost		Lived
5	1½	61½	54	32		30 sec.		Lived
6	1½	70½	56	45		30 sec.		Lived

side to the abdomen 24 minutes later, was moving ataxically 32 minutes after the readmission of oxygen. On the day following the experiment, the animals could not be distinguished in any way from control animals. Moreover, some of the dogs and all of the rats were observed for a number of weeks, and their growth, activity, neurological status and apparent cerebral function were all unimpaired by the prolonged asphyxia. Acute

complete anoxia in the newborn would thus appear to be an "all-or-none"
reaction, resulting in either complete recovery or an immediate fatal
termination. This would seem to indicate that, as in the adult (10, 11),
the heart is more sensitive to asphyxia than is the brain.

DISCUSSION. An unexpected result of this investigation was the ob-
servation that spinal shock does not appear in very young animals during
arrest of the brain circulation as it does in adult animals. A survey of the
available literature has failed to reveal a previous report of this finding.
Since the degree of spinal shock which is produced in animals is less the
lower the species in the evolutionary scale, this is, perhaps, another ex-
ample of ontogeny recapitulating phylogeny. Viewed in this light, one
may, perhaps, consider the generally greater resistance of newborn animals
to anoxia also as an example of this fundamental biological principle,
since lower forms of animals are known to be more resistant to anoxia than
higher forms.

Evidence has been presented that the brain of the very young animal
is very resistant to arrest of its circulation and that this resistance de-
creases rapidly at first and then more slowly with advancing age. The
greater resistance of the young brain has been demonstrated by studies of
loss of function during brain stasis (survival time), recovery of function
after restoration of cephalic circulation (recovery time) as well as the
ability to recover completely following prolonged arrest of circulation
(revival time). A greater resistance to anoxia of the heart, as well as the
brain, has also been indicated by the long survival and revival times of
acute complete anoxia in the newborn. All of these indices of resistance
to anoxia were consistent in illustrating the factor of age. The change in
resistance to anoxia from birth to maturity is of a magnitude beyond the
probable error involved in these experiments, since the resistance of the
newborn as shown by revival time was 400 per cent greater than the adult,
while the resistance of the newborn respiratory center as shown by sur-
vival time was perhaps 1700 per cent greater than the adult.

Previous investigators have established the fact that newborn animals
are more resistant to anoxia than are adults. Reiss and Haurowitz (1),
who limited their study to survival time, found that newborn mice were
more resistant than adult mice, whether the anoxia was produced by car-
bon monoxide, hydrogen, carbon dioxide or hydrogen cyanide, while age
was not a factor in susceptibility to chloroform. Calculating from their
data, the ratio of resistance of newborn to adult for pure carbon monoxide
is 132:1 and for hydrogen cyanide 117:1. Avery and Johlin (2) also
demonstrated the greater resistance of young animals to carbon monoxide
asphyxia. In the course of other studies, incidental observations have
been made on the greater resistance of the young brain to arrest of its cir-
culation by Crile and Dolley (12) and Andreyev (13).

The greater resistance of the brain of the newborn to arrest of circulation is probably dependent on metabolic factors. The basal metabolism of the newborn per unit weight is greater than that of the adult, but per unit body surface is smaller than in the adult (14). Himwich and his co-workers (15, 16) have studied the metabolism of infant brain slices, in vitro, and found that the QO_2 per unit dry weight was the same as that of the adult in the absence of substrate or in the presence of lactate. On the other hand, in terms of dry weight, in the presence of glucose the QO_2 of the brain of infants 1 to 11 days of age was 11.7, while at 12 to 24 days of age it was 13.6, which is the adult level. Per unit *wet* weight of minced brain, the O_2 uptake of the infant was 123 compared with 200 for the adult. This is a much smaller difference than has been noted in respiratory survival time and in revival time of very young and adult dogs in our experiments. The difference in rate of oxygen utilization of the brain of the infant and the adult may thus be a minor factor in the greater resistance to anoxia of the infant brain.

Another factor to be considered in relation to the resistance of the brain to anoxia is the ability of the neurons to obtain energy in the absence of oxygen. Reiss (17) reported that the major factor involved in the resistance of newborn mice and rats to anoxia (carbon monoxide poisoning) was the ability to break down carbohydrate to lactic acid anaerobically. He compared the lactic acid content of the entire body of normal and fatally asphyxiated animals in the newborn and the adult. The increase of lactic acid produced by asphyxia in the newborn was 460 per cent compared to an increase of lactic acid of 23 per cent in asphyxia in the adult animal. Reiss also noted that death occurred from asphyxia in the newborn with a large carbohydrate reserve still present. Moreover, while the animals succumbed much more rapidly to anoxia at 37°C. than at room temperature, the lactic acid levels were the same at the time of death. This indicates that the cause of death may be accumulation of lactic acid. Since the increased resistance to anoxia in the newborn appears to involve the heart and other tissues as well as the brain, it is a reasonable assumption that the infant brain shares in the increased anaerobic glycolysis.

Reiss' (17) figures for lactic acid and our own data for revival time correspond closely. Reiss found that the increase of lactic acid production in asphyxia in the newborn as compared to the adult was 430 per cent. The data presented above on revival time (fig. 1) indicate that the brain of the newborn is approximately 400 per cent more resistant than that of the adult to arrest of circulation. It is interesting to note that Craigie (5) found an increase in vascularity of the rat parietal cortex from birth to maturity of 440 per cent.

Statements appear in the literature without experimental evidence

that the brain of the newborn is *less* resistant to anoxia than the adult
brain.   Thus, in a discussion of paranatal asphyxia, Schreiber (18) states:
"Considerable clinical evidence supports the conclusion that the brain
tissue of an infant can sustain much less oxygen deprivation than can
the adult organ, and therefore, is more readily damaged from this par-
ticular cause than is adult cerebral tissue."   Clinical observations on
asphyxia neonatorum (19) reveal, however, that the newborn infant can
recover completely after severe asphyxia prolonged far beyond what would
constitute fatal asphyxia in the adult.   The great resistance of the respira-
tory center of the newborn is attested to by the prompt response of severely
asphyxiated infants to lobeline.   There seems to be good reason to believe,
therefore, that the brain of the human infant is more resistant to anoxia
than the adult organ.

SUMMARY

1. The young dog is much more resistant to acute asphyxia than is the
adult animal.

2. The brain of the young animal is much more resistant to arrest of its
circulation than is the adult organ.   This has been demonstrated by quan-
titative studies of survival time, recovery time and revival time after
temporary complete arrest of the cephalic circulation.

3. In the newborn, the respiratory center will apparently continue to
function 17 times as long as in the adult during complete arrest of blood
flow in the brain.

4. The ability of the newborn to achieve complete functional recovery
following periods of complete arrest of the brain circulation is approxi-
mately 400 per cent greater than that of the adult.

5. The change with age in resistance of the brain to arrest of its circula-
tion follows a hyperbolic curve, decreasing rapidly in the first month and
more gradually later.   The resistance is diminished to the adult level at
the age of four months.

6. Spinal shock does not supervene during arrest of the brain circula-
tion in young dogs.

7. The relation of the increased resistance to brain anoxia in young ani-
mals to changes with age in metabolism and vascularity of the brain is
discussed.

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HORMONAL INFLUENCES ON CARBOHYDRATE METABOLISM DURING WORK

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The effect of adrenalin on the carbohydrate metabolism during muscular exercise has been studied by Dill, Edwards and de Meio (1935), who found that the RQ after adrenalin injections was considerably higher than in control experiments without adrenalin. They concluded that with the doses of adrenalin used the utilization of sugar was increased. This conclusion has been questioned by Courtice, Douglas and Priestley (1939), who assert that the initial increase in RQ depends on the increase in blood lactic acid rather than on oxidative changes and that a subsequent period with subnormal RQ when lactic acid is being removed brings the RQ for the whole period of accumulation and disappearance of lactic acid to the level of control experiments without adrenalin. As the material published by Courtice, Douglas and Priestley does not, in our opinion, convincingly support this assertion, we have repeated the experiments under standardized conditions and with special care for the control experiments.

The procedure followed is very close to that followed by Courtice, Douglas and Priestley. The work consisted in walking on the treadmill at 5.6 km. an hour at a grade of 8.6 per cent for three hours. Expired air was collected for ten out of every fifteen minutes during the experiments, except during the 30 minutes following the injections when collections were made for four minutes out of every five. Blood samples, venous or capillary, for determination of blood sugar, lactic acid, and ketone bodies, were drawn regularly at thirty-minute intervals, but, whenever necessary, at shorter intervals. Urine was voided before work, just after work, and one-half hour later, and was tested for acetone.

The subjects (A, 32 years, and W, 26 years) came fasting to the laboratory in the morning. They lived on an ordinary mixed diet. Subject W did not change this diet throughout the period of experiments while subject A, about halfway through the period, changed his diet to one considerably richer in carbohydrates. The injection of 1 mgm. of adrenalin chloride (Parke, Davis & Co., 1:1000) was made intramuscularly at the end of the first hour of walking, the first hour of work being used as a control. In further experiments 3.5 units of insulin (solution of zinc-insulin

crystals, Lilly) was given intravenously, either alone or combined with adrenalin chloride intramuscularly or sugar orally.

The air samples were analyzed at the Haldane apparatus, blood sugar determined after Folin and Malmros (1929), lactic acid after Edwards (1938), and blood acetone after Van Slyke and Fitz (1917). One week or more elapsed between performances in order to secure complete recovery.

Control experiments. Control experiments showed that the effects of the work on metabolism, RQ, and blood compounds were very well reproducible as long as the diet was unchanged; in subject A, when at the time of the last experiments a carbohydrate-rich diet was being taken, the only effect found was a slightly higher trend in the RQ than in the first experiments. This effect of the diet on the RQ has been shown recently by Christensen and Hansen (1939), but it is emphasized that the RQ in exercise may be altered by other factors than diet. Unpublished experiments by Dill show that even on a prolonged constant diet considerable changes in the trend of the RQ during constant work may be found, and we had, in

TABLE 1

SUBJECT	CALORIES				CALCULATED RQ PER HOUR			TOTAL CARBOHYDRATE	CALORIES DERIVED FROM CARBOHYDRATE
	1. hour	2. hour	3. hour	Total	1. hour	2. hour	3. hour		
								gm.	*per cent*
W	498	492	507	1,497	0.881	0.833	0.806	170	45.6
AI	536	536	547	1,619	0.884	0.844	0.807	191	47.1
AII	534	542	558	1,634	0.900	0.877	0.837	229	55.9

connection with the present experiments, to give up a third subject, a young negro, because his RQ during work showed large differences from week to week, although his diet was as constant as that of A and W. Subjects A and W showed during the three hours of walking a steadily increasing O_2 intake. In the case of A it rose from about 1.8 l/min. at the start to about 1.9 l/min., and in the case of W from about 1.6 l/min. to about 1.7 l/min. The RQ decreased during the same time from about 0.91 at the start to about 0.80 at end of the work. Neither blood sugar nor lactic acid nor blood acetone showed any considerable changes during the work. (See figs. 1–5.) The total amount of work done and the amount of carbohydrate burned during this work are averaged in table 1.

The carbohydrate burned is calculated from the RQ without regard to the protein metabolized.

The table shows the steady decrease in RQ during the three hours' walking and also the effect on RQ and on carbohydrate utilization of changing the diet with A. (AI before change in diet, AII after change in diet.) The efficiency with which W worked was apparently better than

A's, their weights being about the same, 69 kgm., as the total output of calories shows.

Adrenalin. The effect of injecting 1 mgm. of adrenalin at the end of the first hour as compared with a control experiment is shown in figure 1 for subject AI. The findings on W were very similar. A calculation of the caloric output and the amount of carbohydrate burned in these experiments is shown in table 2.

TABLE 2

SUBJECT	CALORIES				CALCULATED RQ PER HOUR			TOTAL CARBOHYDRATE	CALORIES DERIVED FROM CARBOHYDRATE
	1. hour	2. hour	3. hour	Total	1. hour	2. hour	3. hour		
								gm.	per cent
W	504	516	522	1,542	0.874	0.879	0.793	188	48.9
AI	533	561	535	1,629	0.886	0.885	0.834	225	55.2
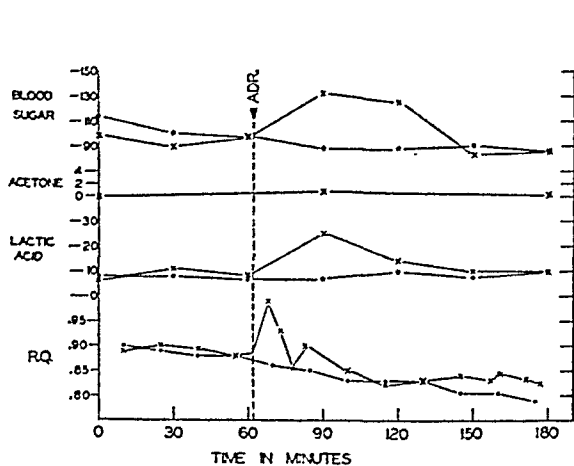
Fig. 1
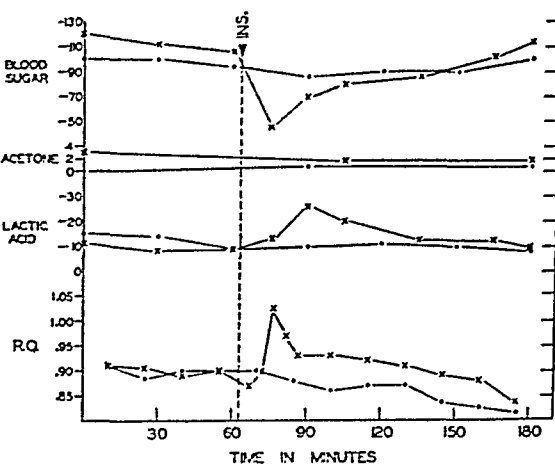
Fig. 2

Fig. 1. Subject A. Blood sugar, lactate, and acetone in milligrams per cent and RQ during three hours' walk. ·———· control experiment, x———x experiment with injection of adrenalin (at *ADR.*).

Fig. 2. Subject A. ·———· control, x———x experiment with injection of insulin (at *INS.*).

A comparison of tables 1 and 2 shows an increased utilization of carbohydrates after administration of adrenalin. The curves of figure 1 show that the increase in RQ immediately following the injection to a certain degree is counteracted by a decreased RQ later on. Part of the increase in RQ is doubtless due to the simultaneous increase in the concentration of lactic acid, which will drive out an equivalent amount of CO_2. As the lactic acid concentration again decreases a corresponding amount of CO_2 must be retained and this will result in an apparently low RQ. In the last

30 minutes of the work the lactic acid concentration in both subjects was normal, and the RQ found in this period can consequently be assumed to be real.   The two hours after the injection of adrenalin must therefore have given the organism time enough to restore the blood and alveolar CO_2, and one is entitled to calculate the amount of carbohydrate oxidized during the three hours' work from the total RQ of the whole period.

The increased RQ following an injection of adrenalin may depend upon: 1, the increased blood sugar level, offering more sugar for the muscles to burn; 2, the increased concentration of lactic acid, favoring oxidation of lactic acid and thus increasing the RQ; 3, the high blood sugar level, stimulating the pancreas to put out more insulin, which, besides affecting the storage of sugar in the liver and in the muscles, might cause the muscles to burn more sugar.   In order to test some of these possibilities, a series of complementary experiments has been carried out, which will be described in the following.

Insulin and glucose.   Figure 2 shows the effect of an intravenous injection of insulin (3.5 units) on blood sugar, lactate, acetone bodies, and RQ in subject A.   The low blood sugars were accompanied by hypoglycemic symptoms (impaired vision and hearing, difficulty in continuing the work, perspiration), later followed by a strange feeling of numbness and cold in the legs.   This feeling soon disappeared, however.   It can be seen that the maximal RQ is found at a time when the lactic acid is still rapidly increasing.   A calculation of the total percentage of carbohydrate calories in these experiments gives 65.8 per cent for AII, 52 per cent for W, compared with 55.9 per cent and 45.6 per cent respectively in the controls.

Figure 2 shows that high RQ's are not necessarily dependent on high blood sugars.   The increase in lactic acid and the high peak on the curve representing the RQ might in this case be due to a secretion of adrenalin evoked by the low blood sugars.   We tried in two ways to avoid these low blood sugar values: We combined the effect of adrenalin with that of insulin, and we gave glucose combined with insulin.

The results of an experiment in which both adrenalin and insulin were given are shown in figure 3 (subject W).   The adrenalin was given 15 minutes before the insulin.   Calories derived from carbohydrates in AII were 57 per cent and in W were 51 per cent, compared with 56 per cent or 46 per cent in the controls.   In the case of AII it might be remarked that the RQ in the first hour was unusually low (0.862 compared with 0.900 in the controls).

When 100 grams of glucose were given 20 minutes before the injection of insulin, the results came out as shown for W in figure 4.   The percentage of calories derived from carbohydrates was 64.8 per cent for AII, and 61.6 per cent for W, a considerable increase compared with the controls, 55.9 per cent and 45.6 per cent.

In a last experiment the effect of 100 grams of glucose alone was tested. Figure 5 shows the result on W. Calories derived from carbohydrate were 59.8 per cent by AII and 55.9 per cent by W.

The two subjects showed no ketonuria in rest before the walk. After the three hours' walk W's urine showed a slight positive nitroprusside reaction in the control experiments and in the experiments where adrenalin was given—none in the other. One-half hour after the work, however, the test was strongly positive for W in the same experiments and faintly
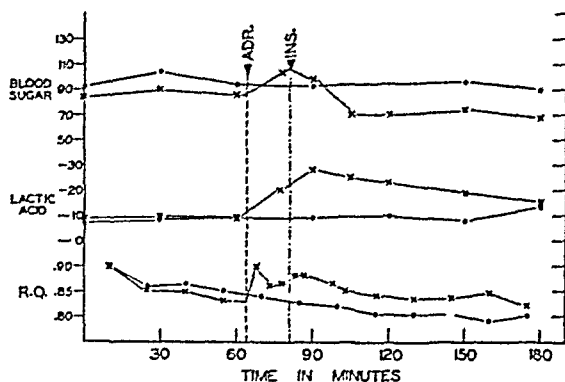
Fig. 3
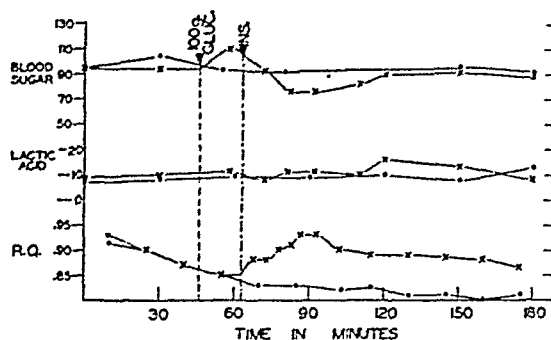
Fig. 4

Fig. 3. Subject W. ·———· control, x———x experiment with injection of adrenalin (at *ADR.*) and insulin (at *INS.*).

Fig. 4. Subject W. As formerly; at first mark 100 grams of glucose were given orally; at second, insulin injection.
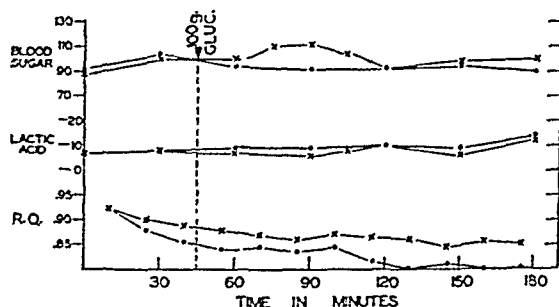
Fig. 5. Subject W. One hundred grams of glucose given at mark.

positive for A. The blood acetone showed no alterations during the three-hour walk and neither adrenalin nor insulin seemed to change its concentration in the blood. A slight increase in blood acetone one-half hour after the walk was often observed. Figures 1 and 2 and table 3 give the data obtained.

DISCUSSION. The results of the experiments in which adrenalin was given intramuscularly showed in accordance with the results of Dill, Edwards and de Meio that adrenalin increases the RQ during muscular

exercise.   Courtice, Douglas and Priestley ascribed this result to the effect of the lactic acid on the CO_2 of the blood and presented curves which show a slight compensatory decrease in RQ later during the time when lactic acid is removed.   As our experiments, however, last at least 30 minutes longer than necessary for complete removal of excess lactic acid, it is possible to determine the true RQ for the entire period and thereby the percentage of the total caloric output which is derived from carbohydrates. Tables 1 and 2 show that this is increased after administration of adrenalin. The apparent contradiction between these results and those of Courtice, Douglas and Priestley might be due to the assumption which those authors apparently made: that the RQ at start and at end of their work should be the same.   This might be the case with easy work corresponding to 210 kgm. per min.   It is very improbable in work carried on at the rate of 700 kgm. per min.   (We have never observed a constant RQ in subject A

TABLE 3

Acetone bodies in blood calculated as milligrams of acetone per 100 cc.

	SUBJECT A				SUBJECT W			
	Immediately after work		Thirty minutes later		Immediately after work		Thirty minutes later	
	Urine	Blood	Urine	Blood	Urine	Blood	Urine	Blood
Control.....................	0	1.0	(+)	2.8	+	3.8	+++	6.8
Adrenalin..................	0	0.5	+	1.9	(+)	5.2	+++	4.6
Insulin.....................	0	1.7	0	2.0	0	4.2	+++	5.2
Adrenalin and insulin........	0	1.6	+	0.5	0	3.1	0	1.2
Glucose and insulin..........	0		0		0		0	
Glucose....................	0		0		0		0	

or W, nor in other fasting subjects working at about this rate.)   Unfortunately, they do not publish a two-hour control curve for this rate of work, but their first three values seem to indicate that there should be a slope in the RQ curve as there is in the corresponding curves in our control experiments.   In such a case there will be no contradiction between the two series of experiments.

It is generally assumed that adrenalin has at least two different effects on the carbohydrate metabolism: It accelerates the breakdown of liver glycogen into glucose and it increases the transformation of muscle glycogen to lactic acid.   The first of these processes will cause a rise in the blood sugar which in its turn might well evoke a compensatory increased secretion of insulin.   The effect on the RQ might therefore very well be an indirect insulin effect.   On the other hand, a breakdown of muscle glycogen will give rise to an increased concentration of lactic acid, and the removal of this, by partial or total combustion in muscles or liver, will raise the RQ.

A merely passive increase in the combustion of carbohydrates by the muscles caused by the higher blood sugar level seems very unlikely in view of the experiments of Christensen and Hansen (1939) and others. The lack of simple dependence between carbohydrate oxidation and blood sugar concentration is proved by the increase in RQ after insulin, when the blood sugar is lowered (fig. 2).

The effect of insulin on the carbohydrate metabolism during work can be divided into 1, a direct effect on liver glycogen, blood sugar, and on the sugar oxidation in the muscles, and 2, an indirect effect on the muscle glycogen and the lactic acid concentration. This second effect can be suppressed if glucose is given simultaneously in such a way as to prevent too large a fall in the blood sugar concentration (fig. 4). It might therefore well be assumed that the low blood sugar can evoke an "emergency" secretion of adrenalin (or pituitary hormone), which causes the breakdown of muscle glycogen into lactic acid. This explanation will account for both the increased lactic acid concentration and the very high values of RQ in figure 2 shortly after the injection of the insulin. The combined effect of adrenalin and insulin is practically the sum of their single effects (compare with Courtice, Douglas and Priestley). It is remarkable that the blood sugar stays low and the lactic acid relatively high for a longer time than they would after a single injection of either hormone. The increased RQ might be due to both an increased oxidation of sugar and of lactic acid.

Since glucose and insulin given simultaneously have no effect on blood lactate and—at least in W—little effect on blood sugar, the increase in the RQ in this case depends on the direct effect of insulin on carbohydrate oxidation. While glucose alone in our two subjects increased the blood sugar but slightly, the RQ declined less than usual during the work.

The ketonuria found one-half hour after the end of the work corresponds to the ketonuria which Courtice, Douglas and Priestley found in their investigation. While the organism has in insulin a very effective and fast-working hormone for restoring mobilized carbohydrate, the removal of acetone bodies apparently has to be effected by complete oxidation. Under the conditions of our experiments acetone formation did not exceed its rate of oxidation so long as work was going on, but when work stopped formation exceeded removal, blood acetone concentration increased, and some spilled over in the urine. It is worth emphasizing that the statement frequently found in textbooks that adrenalin impairs the carbohydrate utilization does not apply to exercise. This idea was based on the erroneous report that blood acetone increases after adrenalin (compare Dill, Johnson and Daly, 1939).

A comparison of the here-mentioned effects of an injection of adrenalin with the effects of an injection of insulin on blood compounds and RQ

during work shows that only their effect on the blood sugar seems to be different.   Adrenalin and insulin given simultaneously have no pronounced effect on the blood sugar, and an antagonism between the two hormones with respect to the blood sugar regulation is also commonly assumed. As the insulin, when glucose is given simultaneously so that a deep fall in blood sugar is avoided, does not affect the blood lactate, it is reasonable to assert that this effect of insulin is indirect, caused by an "emergency" secretion of adrenalin.   The rise in RQ after insulin is, no doubt, due to a direct effect of insulin on the carbohydrate utilization, whereas the corresponding effect of adrenalin might be interpreted otherwise; the removal of lactic acid is certainly accompanied by an oxidation of at least a part of the produced lactic acid, and this might cause the rise in the RQ found after injection of adrenalin.

SUMMARY

The effect of injection of adrenalin, insulin, or both, and of administration of glucose orally on the carbohydrate utilization during muscular exercise has been studied in two normal subjects.   Compared with control experiments without injections the effect 1, of adrenalin was: increased RQ, blood sugar, and blood lactate; 2, of insulin: increased RQ, decreased blood sugar, increased blood lactate; 3, of adrenalin and insulin simultaneously: increased RQ, small fluctuations in the blood sugar level, increased blood lactate; 4, of glucose: increased RQ and blood sugar, no changes in blood lactate; 5, of glucose and insulin simultaneously: increased RQ, fluctuations in blood sugar, no changes in blood lactate.   Neither hormone showed any effect on the blood acetone.   The increased RQ following the injection of either hormone must be taken as a sign of increased carbohydrate combustion.

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FURTHER OBSERVATIONS ON TOTAL CHLORIDE CONTENT

The Relationship Between Body Fat and Body Chloride

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In a previous communication an indirect method for estimating the total chloride content of animals following the administration of sodium bromide was described (1). Attention was directed to the observation that within a given species of animal no inconsiderable variation in the chloride content per kilogram body weight might occur. Of the probable reasons for this variation in total chloride content, one possibility—namely, that the variation in total chloride might be related to variations in total fat content—seemed especially worthy of further investigation.

It was considered probable that since fat may contribute considerably to the body weight of an animal and but insignificantly to the chloride content of the animal, all normal animals of a given species might be found to contain essentially constant quantities of chloride for each kilogram body weight provided the weight was expressed on a fat-free basis.

The experiments to be reported were undertaken primarily to test this hypothesis. Moreover, it was felt that further information on the total water and total fat content of dogs, as of necessity directly determined in the course of this work, would be desirable.

METHODS. This study involved determinations of total chloride, total water, and total fat content of dogs.

Determination of total chloride content. The procedure for determining the total chloride content of animals has been described previously (1). Identical methods were employed in this study.

Determination of total water and total fat content. Following the period of equilibration with sodium bromide and withdrawal of the sample of blood for analysis, the contents of the gastrointestinal tract were removed and discarded, and the entire animal was minced using meat cleavers. The minced tissue was spread out, single layer deep, in large pans and placed into a drying oven maintained at 105°C. for 36 to 48 hours. The difference between the initial weight of the animal and the weight of the dried tissue permitted calculation of the water content.

The dried residue was then transferred without loss to a large glass

:stoppered specimen bottle and extracted successively with ether and alcohol. Four to six days of extraction with each substance were required to remove the fat. During this time the ether and alcohol were renewed frequently and recovered by distillation. Total fat content was determined as the difference in weight before and after fat extraction.
⊦ In these experiments care was taken to select only adult dogs.

TABLE 1

Total chloride content of dogs before correction for fat content

DOG	[Cl⁻ + Br⁻]	[Br⁻]	[Cl⁻]	$\frac{[Cl⁻]}{[Br⁻]}$	MOLS Br⁻ INJECTED	MOLS Cl⁻ CALCULATED	WEIGHT	CHLORIDE PER KGM.
	mM/liter of serum							
							kilograms	*grams*
1	130.5	33.2	97.3	2.93	0.0677	0.1984	5.52	1.27
2	129.8	34.8	95.0	2.73	0.0689	0.1880	5.15	1.29
3	142.5	40.0	102.5	2.56	0.0678	0.1735	4.67	1.32
4	131.7	31.6	100.1	3.17	0.0594	0.1884	5.15	1.30
5	134.0	38.4	95.6	2.49	0.0593	0.1476	4.55	1.15

TABLE 2

Total fat, total water and total chloride content of dogs; the corrected values are based upon the fat-free body weight

DOG	TOTAL FAT	TOTAL WATER		CHLORIDE PER KILOGRAM	
		Uncorrected	Corrected	Uncorrected	Corrected
	per cent	*per cent*	*per cent*	*grams*	*grams*
1	6.9	68.2	73.2	1.27	1.37
2	13.1	65.6	75.5	1.29	1.48
3	4.8	69.0	72.5	1.32	1.39
4	7.1	68.9	74.2	1.30	1.39
5	14.8	62.2	73.0	1.15	1.35
12	14.7	62.2	73.0		

RESULTS AND DISCUSSION. In table 1 are shown the values for the chloride content of a series of dogs estimated from determinations of serum chloride and bromide.

It will be observed that all the animals in this series fall within the range previously reported for dogs (1.0–1.4 gram Cl/kgm. body weight). In view of our previous observations that the chloride content might show marked variations among dogs, the agreement in chloride content of the dogs of the present series was entirely unexpected. Consideration of the data presented in table 2, however, leads to the conclusion that this agreement is explicable most satisfactorily on the basis of the fat content of the dogs.

It will be noted in this table, that the values expressing the chloride content per kilogram of fat-free body weight are in essential agreement. Furthermore the data show that even without correcting for body fat agreement in chloride content may be expected if the fat content of dogs is approximately equal. Thus of the four dogs showing similar chloride values before correcting for fat three of the dogs (nos. 1, 3 and 4) were found to contain fat in amounts varying only between 5 and 7 per cent. Lack of close correlation between fat and chloride content is shown only by dog 2. We are inclined to attach significance to the fact that the corrected water content of this animal is higher than that of all the other animals. It should be clear that if water retention or edema were present in an animal the apparent relationship between fat and chloride would vanish.

Of considerable interest is the observation that the total water content as directly determined in a series of ten dogs (cf. also table 3) varied only between 60 and 69 per cent with an average of 65 per cent. This agrees

TABLE 3

Ratio of skeleton weight to body weight in dogs

DOG	WEIGHT	WATER CONTENT	FAT CONTENT	WEIGHT OF SKELETON	SKELETON WEIGHT BODY WEIGHT†
	kgm.	*per cent*	*per cent*[*]	*grams*	*per cent*
14	5.95	66.0	10.1	333	6.2
15	6.50	63.4	13.7	451	7.6
16	4.97	68.4	6.8	254	5.5
17	5.90	60.6	17.6	376	7.7

* Estimated assuming fat-free water per cent = 73.6.
† Fat-free.

with the recent findings of Gregersen and Painter (2) who used indirect methods for determining water. Particularly striking, however, is the slight variation in the corrected water values based upon fat-free body weight. Indeed it would seem that approximations of body fat could be made from indirect determinations of total body water in the normal intact animal.

The basis for the relative constant chloride and water content of fat-free normal dogs would seem to lie in a, a relatively constant chloride and water content of individual tissues and organs, and b, a relatively constant ratio of tissue or organ weight to total body weight. For fat-free striated muscle Hastings and Eichelberger (3) have shown in a large series of dogs that the water content is 76.5 ± 0.6 per cent. The chloride content of striated muscle and blood serum also was found to vary little. Their observations taken together with the finding that the total, fat-free, water content is constant is presumptive evidence that the proportion of various

tissue weights to body weight is constant. This conclusion is the more probable since the water content of different tissues varies over wide limits (4).

In a series of four dogs a direct determination of the ratio skeleton weight:body weight was made. The skeleton was obtained entirely free of muscle and connective tissue by placing the partially cleaned bones of the animal in a steam macerator. After maceration the remaining flesh virtually fell from the bones. The cleaned bones were then fat extracted, dried, and weighed. In table 3 are shown the results of these experiments.

Insofar as the ratio of skeleton to fat-free body weight shows agreement in the four dogs the data lend support to the suggested explanation of the constancy in chloride and water content.

CONCLUSIONS

Evidence derived from simultaneous determinations of body chloride, body fat and body water indicates that the previously reported marked variation in chloride content per kilogram body weight in dogs is due, in the main, to variations in fat content. Expressed in terms of fat-free body weight the chloride content of normal dogs varies slightly from the average value of 1.4 gram Cl/kgm. body weight. This latter value does not invalidate the previously reported average chloride value which was obtained on dogs without correcting for body fat.

The total water content of dogs was found to be 74.0 ± 1.5 per cent of the fat-free weight or 64.8 ± 4.2 per cent of the total body weight.

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THE ACTION OF WATER MOCCASIN VENOM ON THE ISOLATED FROG HEART

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The action of various snake venoms on isolated frogs hearts has been studied by many investigators. Cobra venom has been so studied by Elliott (1901), Gunn (1912), Cushney and Yagi (1918), Gunn and Heathcote (1921), Meurling (1935), and others. Magenta (1922) studied the effects produced by venoms from fifteen species of poisonous snakes, by the Straub method. Unfortunately his results are published in such abbreviated form that the effects of individual venoms cannot be determined.

Among the venoms Magenta used was that of the water moccasin, Agkistrodon piscivorus Lacépède. The action of the venom of this species of snake has also been studied by Essex and Markowitz (1930), and by Essex (1932), using hearts of rabbits. When a Ringer-Locke perfusion fluid to which dried venom was added to make a final dilution of 1:250,000 to 1:500,000 was used, the hearts were incapacitated in ten to fifteen minutes, indicating a much greater cardiac toxicity than is possessed by the venom of the timber rattlesnake, Crotalus horridus. In view of the small amount of study of the water moccasin venom, the author determined to investigate the venom, especially because of the frequent statement that this venom is essentially similar to that of the North American rattlesnakes, e.g., Noguchi (1909), Essex and Markowitz (1930), Essex (1932).

In all experiments, isolated hearts of spring or summer frogs were used. A modified Straub method, "constant drip," was necessary. The modifications were: 1, arrangement of the reservoir containing the perfusion fluid so that a constant stream of fluid ran into the cannula; 2, a cotton wick at the upper end of the cannula, which prevented formation of a

large meniscus, with consequent alteration of the diastolic filling; it also distributed the overflow evenly over the exterior of the heart. The rate of flow of the perfusion fluid was such that one or two drops were expelled at each systole.

The venom was collected in the latter part of the summer of 1938, by the well-known method of "milking." The collected venom was dried under an electric fan, ground in a mortar and the various batches mixed to form the stock dry venom. The stock represented ten milkings from twenty-eight snakes. Mitchell and Reichert (1886), and Macht (1933 and 1937) have shown that any alteration in such dried venoms is in the direction of some loss in potency, if there be any change.

The perfusion fluid used in all experiments was Ringer solution of the following composition, expressed in millimoles per liter: NaCl 112.20, KCl 1.88, CaCl$_2$ 1.04, NaHCO$_3$ 2.38. To this was added enough weighed dry venom to give the desired dilution.

The contractions of the hearts were recorded on a kymograph by means of a light heart lever, fastened to the tip of the ventricle by a fine silk thread. For each dilution used eight[1] hearts were run. In the control group of eight hearts, one failed completely in 180 minutes. This duration was considered as maximum in all subsequent experiments. In experiments with venom, the hearts were run in the Ringer solution until they had become physiologically constant; at this time the cannula was filled with the Ringer-venom solution and the drip started.

Each record was measured for amplitude of contraction. For each, the initial amplitude was arbitrarily considered as ten centimeters, an approximation of the actual average amplitude. These results were averaged for each experimental group, i.e., controls, 1:1,000, 1:10,000, 1:100-000, and 1:1,000,000 venom-Ringer solutions. The results are shown as figure 1.

The control hearts run with Ringer solution showed no stimulation and a decrease in amplitude of 43 per cent in 180 minutes; the hearts subjected to 1:1,000 venom in Ringer showed practical standstill in 18 minutes and no stimulation throughout the experiment. When venom was used in a concentration of 1:10,000 there was a brief period of increased amplitude (maximum: 43 per cent; duration: 7 minutes) succeeded by rapid decrease to zero in 24 minutes. The solution of venom 1:100,000 produced a maximum increase of 8 per cent; the duration of the stimulating action was 17 minutes absolute or 43 minutes when compared with the controls. Hearts run with 1:1,000,000 venom-Ringer solution showed an average maximum stimulation of 11.4 per cent and remained more effective than the controls throughout the entire 180 minutes.

[1] The group run in 1:100,000 venom consisted of seven hearts.

The effects on heart rate of the Ringer solution and the various venom solutions are shown in figure 2. The rate of beating of the controls remained rather uniform, i.e., O. Frank's law was applicable, the changes
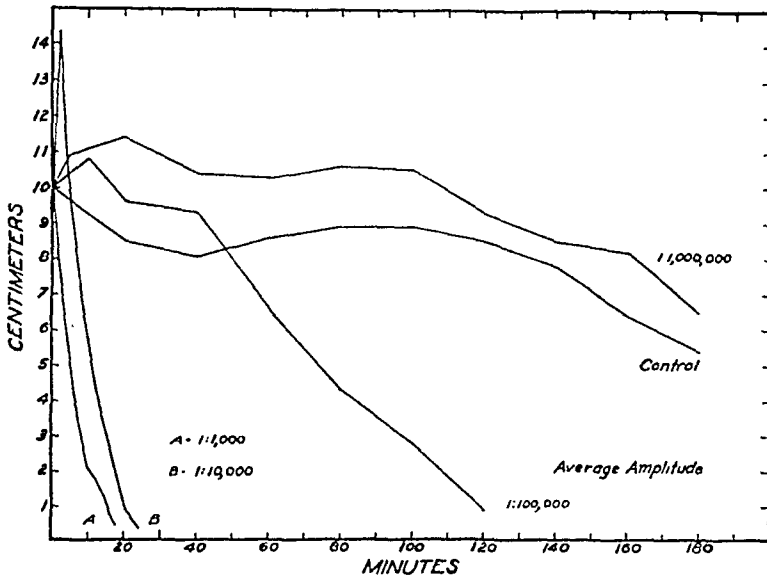
Fig. 1. Average contraction amplitudes of control hearts and those subjected to venom-Ringer solution.
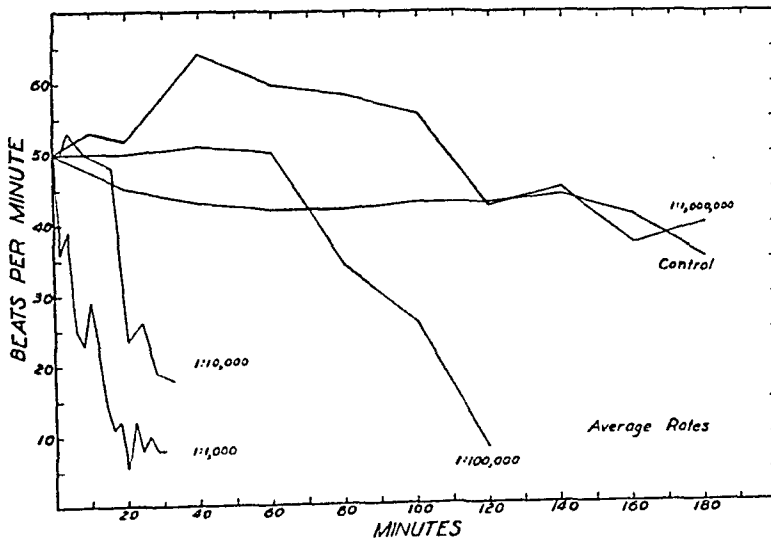
Fig. 2. Average rates of control hearts and those subjected to venom-Ringer solution.

in rate being in general inversely proportional to the changes in amplitude. In 180 minutes the rate of the controls decreased 35 per cent. Marked slowing or complete stoppage resulted from the application of 1:1,000 venom solutions; partial recovery followed. With 1:10,000 venom solutions

there was first marked slowing, followed by a slight increase in rate; this in turn gave way to rapid failure of contraction. The original slowing could be abolished by atropine. In 1:100,000 venom solutions, three of the hearts showed initial slowing, three initial stimulation, and one showed rapid failure with no recovery. The effect of averaging the results is to obscure these effects on the graph. Hearts treated with 1:1,000,000 venom solution showed in several instances very slight initial slowing. There followed great increases in rate in three hearts, moderate increases in three, and in two there was little change in rate.

Shown in figure 3 are the effects of the various concentrations of venom on the output of the hearts; the output of the controls is included. The curves are the products of the amplitude from figure 1 and the rate from figure 2. The measure is relative, but agrees well with several experi-
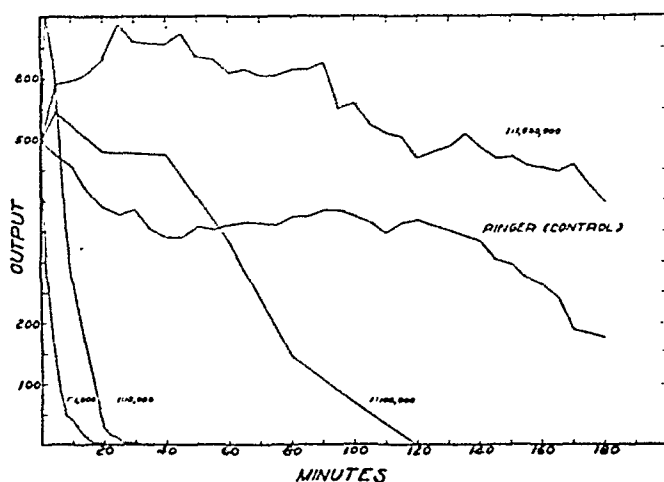
Fig. 3. Average output of control hearts and those subjected to venom-Ringer solution.

ments where actual volume changes were measured. Because of the reciprocal relationship between amplitude and rate, the product of the two is a more reliable measure of effect than either alone. The output of the 1:1,000 hearts fell to zero in 20 minutes; that of the 1:10,000 hearts in 32 minutes and that of the 1:100,000 hearts in 120 minutes. The control hearts showed a 65 per cent loss of efficiency in three hours; the hearts treated with 1:1,000,000 venom solution suffered a loss of only 20 per cent in the same time. The first two solutions were severely cardiotoxic; the 1:1,000,000 solution was beneficial; the 1:100,000 solution produced stimulation, followed by rapid failure.

The order of failure in the hearts was first the ventricle, then the atria, last the sinus venosus. The ventricle stopped in complete contraction in most of the hearts. In those treated with high dilutions of venom the

contraction was sometimes incomplete; mechanical stimulation induced complete contraction. The muscle became whitish and opaque, with a "cooked" appearance. The atria failed in wide dilatation. They were also opaque. The sinus venosus remained clear and beat for some time after failure of the atria and ventricle.

Dissociation occurred only rarely except in the terminal stage of ventricular activity, where complete ventricular failure was imminent. This is in contradiction to Magenta's (1922) results. When dissociation did occur, it was of the type of dissociation with capture.

Extra systoles were rather common. Muscular irregularities frequently occurred, accompanied by local regions of contracture in the ventricle. These effects disappeared as the elasticity of the myocardium decreased, a constant effect with all venom solutions except the weakest, as shown by progressive failure of rebound of the heart lever.

The venom acted upon the vagal endings or the ganglion cells of the heart to produce slowing. This slowing could be almost completely abolished by atropine. In several of the hearts, especially those treated with high dilutions of the venom, the release from vagal effects was quite sudden; in one case the rate doubled in less than one minute. The probable site of action is the ganglion cells, which are first stimulated, then depressed. This is in accordance with the stimulation and subsequent depression of the myocardium seen above.

An outstanding effect of the venom solutions was the tremendous increase in the permeability of the atria. These became so permeable that the "constant drip" method was necessary to keep the Straub cannula filled. Obviously the endocardium was severely damaged. There appeared to be no fluid loss through the ventricle, probably because the much heavier, more dense muscle prevented fluid escape.

Because of the many obvious muscular effects and the constant dialyzing action of the "constant drip," the author believes the results to be due to direct action of the venom on the myocardium, endocardium, and intrinsic ganglia, rather than to the action of lysolecithin and histamine produced by the action of the venom on the tissues. This view is in agreement with that of Essex (1932) and receives correlative support from the work on rattlesnake (Crotalus horridus) venom of Dunn (1934); Essex and Markowitz (1930b); Dragstedt, Mead, and Eyer (1938); and from the demonstration of such direct effects of snake venoms on protoplasm by Lepow (1938) and the importance of the change of lecithin into lysolecithin on the properties of surface films as shown by Hughes (1935). The hypothesis that the effects of the venom are caused by the lysolecithin and histamine produced, as stated by Belfanti (1925), Magenta (1922), Houssay (1930), and Houssay, Negrete, and Mazzocco (1933)

seems unnecessarily complex. This aspect of the problem is well reviewed by Kellaway (1939). The author believes the venom acts directly and that the lysolecithin and histamine are by-products of such direct action, rather than being the causative agents of the tissue changes.

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SUMMARY

The venom of the water moccasin, Agkistrodon piscivorus Lacépède, acts directly on the endocardium, myocardium and intrinsic ganglia of the isolated frog heart.

In 1:1,000 concentration, the venom has a depressant action on the heart; in 1:10,000 and 1:100,000 concentrations there is preliminary stimulation, followed by failure.

Concentrations of 1:1,000,000 are stimulating; the hearts exceeded the efficiency of the controls throughout the experimental period.

The ventricle failed first; stoppage was usually in complete contraction, although with weak venom solutions it might be in partial contraction. There were muscular irregularities and contractures with all but the weakest solutions.

The atria were rendered highly permeable, permitting rapid escape of fluid through their walls. Failure was in extreme dilatation, occurring after that of the ventricle.

The sinus venosus appeared to be quite refractory to the venom action and failed last.

Dissociation rarely occurred, except in terminal period of ventricular action, where it presaged complete failure in short order.

Stronger venom solutions slow the heart initially by action on the vagus mechanism, probably the ganglion cells. This slowing can be almost completely abolished by atropine.

After failure both the atria and ventricle were opaque and whitish; they looked "cooked."

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THE PRESSOR RESPONSE TO ADRENALIN IN THE COURSE OF TRAUMATIC SHOCK

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The theory that hypersecretion of adrenalin is responsible for the fall in blood pressure in traumatic shock, first proposed by Bainbridge and Trevan (1), has not been widely accepted. In recent years, this theory has been revived by Freeman (2) and Cannon (3). Since Lorber, Kabat and Welte (4) have confirmed the importance of the nervous factor in traumatic shock, it seemed possible that reflex hyperactivity of the sympathetic nervous system might be the mechanism by means of which afferent nerve impulses produce shock. The realization that the evidence supporting the adrenalin theory was of an indirect character, not having involved experiments on animals in shock, prompted an investigation of the rôle of adrenalin in shock.

I. *Traumatic shock resulting primarily from afferent nerve impulses.* A simple technique was devised to produce traumatic shock, involving primarily the nervous factor. Cats were anesthetized with chloralose in doses of 80 mgm. per kilogram of body weight administered intravenously. The hair on the hind legs and lower abdomen was removed and these regions were tightly wrapped with adhesive tape. The leg to be traumatized was bandaged with two layers of adhesive tape and, following trauma, another layer of tape was added. The pelvis was bandaged to the level of the fifth lumbar vertebra. The leg was then maintained in moderate elevation to facilitate venous return. By these simple procedures, it was possible to minimize local fluid loss in the traumatized limb. Blood pressure was recorded continuously on a kymograph by means of a mercury manometer connected to a cannula in the common carotid artery. Chlorazol fast pink, injected into the tubing just above the cannula in 0.5 cc. quantities every half hour, was used as the anticoagulant (5). In all experiments, tracheotomy was performed and the airway inspected repeatedly to be certain that it was clear.

Trauma consisted of 100 blows to the hind leg, which were sufficient to produce multiple fractures of the long bones. The course of the changes in blood pressure following trauma is illustrated in figures 1 and 2. In most instances, the blood pressure rose sharply during trauma. Following

this, the blood pressure fell in every experiment, finally resulting in the death of the animal. In eight experiments, the average survival following leg injury was $2\frac{1}{2}$ hours, while the range of survival was 70 minutes to longer than 7 hours. After termination of the experiment and an autopsy, the tape was removed and the hind quarters carefully separated and weighed to determine the local fluid loss (6). The average fluid loss in the traumatized limb was 0.65 per cent of the body weight with a range from zero to 1.85 per cent of the body weight. There was no significant relation between the magnitude of the fluid loss and the duration of survival. The loss of fluid was small enough in relation to the total blood volume in most instances so that the local fluid loss could be considered of little significance in the etiology of the shock in these experiments (7).

In order to be certain that the nerve impulses from the traumatized limb constituted the essential factor in the production of shock, three control experiments were performed. Fluid loss was minimized by bandaging the limbs with tape, while the nervous factor was eliminated by acute section of the spinal cord at the level of the first lumbar vertebra. Trauma in these animals failed to produce shock or even a significant fall in blood pressure. In one cat, the blood pressure initially was 131 mm. Hg and eight hours after trauma, at which time the experiment was terminated, was 122 mm. Hg. In another experiment, the blood pressure at the beginning was 142 mm. Hg and the final blood pressure seven hours after trauma was 120 mm. Hg. In the third cat, blood pressure at the start was 132 mm. Hg and $4\frac{1}{4}$ hours later was 136 mm. Hg, when the pressure fell suddenly to zero and the animal died of massive hemorrhage at the site of the cord section. These experiments indicate that toxic factors are not paramount in the etiology of this type of traumatic shock and that elimination of significant local fluid loss and of nerve impulses from the site of injury prevents the development of shock.

It has also been possible to confirm the results of Lorber, Kabat and Welte (4), who emphasized the relationship of the depth of general anesthesia to the susceptibility of the animal to traumatic shock. In this experiment, only 33 mgm. per kilogram of chloralose was administered. After a fairly rapid fall of blood pressure to shock level, the pressure rose gradually but steadily over a number of hours. About four hours after trauma, an additional 33 mgm./ kilo of chloralose was administered intravenously, and from then on the blood pressure fell rapidly and the cat expired within an hour. The fluid loss into the traumatized limb was negligible. Since the total dose of chloralose in this experiment was still below the usual anesthetic dose (only 67 mgm./kilo), one can only conclude that the administration of the anesthetic following trauma greatly increased the susceptibility of the animal to traumatic shock.

II. *Pressor effects of physiological doses of adrenalin in the course of shock.*

In eight cats under chloralose, with fluid loss minimized as described above, adrenalin was injected intravenously about every fifteen minutes before and after trauma to the hind leg. The external jugular vein was cannulated and connected to a burette containing warm Ringer's solution. Adrenalin in doses of 0.005 mgm. or 1/20 cc. of 1:10,000 solution was in-
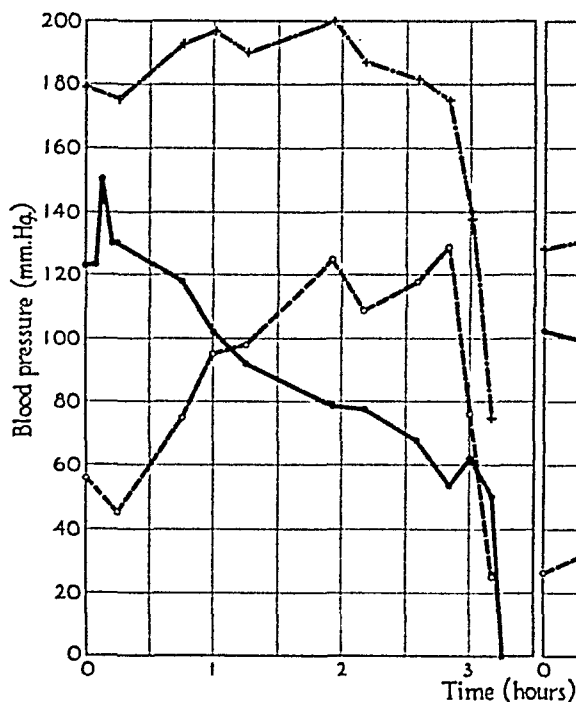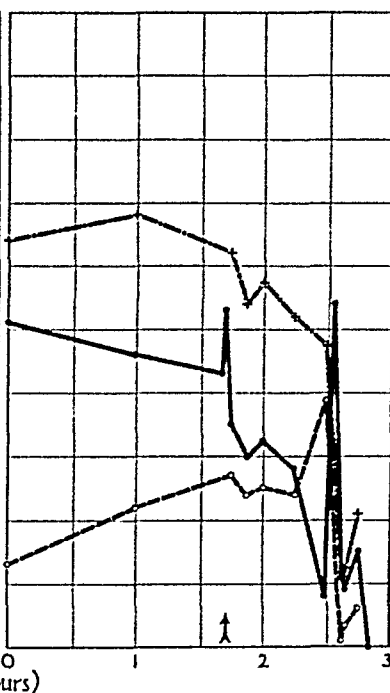
Fig. 1   Fig. 2

Fig. 1. Influence of adrenalin on traumatic shock (expt. 4). The solid line represents the effects of trauma on the blood pressure. The lower broken line represents the rise in blood pressure produced by intravenous injection of 0.005 mgm. of adrenalin at regular intervals. The upper broken line with crosses represents the level to which adrenalin elevated the blood pressure. The arrow indicates the time of trauma. Fluid loss in the injured limb was 0.8 per cent of the body weight.

Fig. 2. Influence of adrenalin on traumatic shock (expt. 7). The solid line represents the effects of trauma on the blood pressure. The lower broken line represents the rise in blood pressure produced by intravenous injection of 0.005 mgm. of adrenalin at regular intervals. The upper broken line with crosses represents the level to which adrenalin elevates the blood pressure. The arrow indicates the time of trauma. Fluid loss in the injured limb was 0.5 per cent of the body weight. The marked rise in blood pressure before death was spontaneous.

jected into the rubber tubing just above the cannula with a 1 cc. tuberculin syringe and was then washed in with 5 cc. of Ringer's solution.

The most striking finding was the sudden marked decrease or complete disappearance of responsiveness to adrenalin just before death. This was observed in every experiment which resulted in fatal shock (figs. 1, 2). This marked decrease in responsiveness to adrenalin was usually evident

within 12 minutes of a good response to the drug and in one case was seen six minutes after an excellent response.   The cats died 2 to 14 minutes

TABLE 1

Terminal loss of responsiveness to adrenalin

EXPERIMENT	TIME	BLOOD PRESSURE BEFORE	BLOOD PRESSURE DURING ADRENALIN	RISE IN BLOOD PRESSURE	REMARKS
1	2:27	77	108	31	
	2:45	62	62	0	
	2:55	0			Death
2	3:15	92	131	39	
	3:30	88	108	20	
	3:40	56	56	0	
	3:45	0			Death
3	10:11	60	106	46	
	10:20	66	66	0	
	10:24	0			Death
4	4:35	54	175	129	
	4:45	62	138	76	
	4:55	50	75	25	
	5:00	0			Death
6	6:27	85	153	68	
	6:40	75	75	0	Blood pressure continues to fall
	6:41	58	Less	0	
	6:42	0			Death
7	4:45	16	95	79	
	4:46	42			Spontaneous increase in blood pressure
	4:48	108			Marked retraction of n.m. and dilatation of pupils
	4:51	22	24	2	
	4:52	18	25	7	
	4:58	30	42	12	
	5:05	0			Death
8	7:47	108	152	44	
	8:07	108	143	35	
	8:20	79	99	20	
	8:23	0			Death

after the failure of adrenalin to elevate the blood pressure.   The data on this terminal loss of responsiveness to adrenalin are presented in table 1.

It is interesting to note that the level of blood pressure at the time of failure of the adrenalin response varied from 75 to 22 mm. Hg. Another indication that the level of blood pressure did not determine the responsiveness to adrenalin is the observation that an excellent response to the drug was obtained at a blood pressure of 16 mm. Hg while a significantly decreased response was noted at a pressure of 88 mm. Hg.

A point of considerable interest is the fact that a slight or marked spontaneous rise in blood pressure immediately preceded the sudden decrease in responsiveness to adrenalin in some cases (figs. 1, 2). For some time before this, the blood pressure had been falling rather steadily. This sudden brief terminal rise of blood pressure was noted in three of the seven experiments which terminated fatally (3, 4, 7, table 1). The nictitating membranes, which had been quite relaxed throughout the post-traumatic period, contracted completely at the time of this brief rise of blood pressure. The pupils were also observed to become widely dilated at this time. The nictitating membranes then remained completely contracted and the pupils dilated until the cat expired. Terminal contraction of the nictitating membranes and dilatation of the pupils was a feature of all cases of fatal shock.

Excluding the terminal failure of adrenalin to affect the blood pressure, the magnitude of the elevation of blood pressure resulting from adrenalin administration was greater during shock than before trauma. As the blood pressure fell with developing shock, the rise, induced by adrenalin, became progressively more marked (fig. 1). Also, the more rapid the fall of blood pressure, the sharper the increase in magnitude of the adrenalin response (figs. 1, 2). In cases where there were significant fluctuations in the blood pressure record following trauma, the changes in the adrenalin response generally bore a reciprocal relation to the changes in blood pressure.

An examination of the absolute level to which the blood pressure rose as a result of adrenalin administration is also of interest. In two experiments, the level to which adrenalin elevated the blood pressure was higher for a time during shock than before trauma (fig. 1). In two other experiments, the level of adrenalin elevation fell sharply soon after trauma and was far below the pre-traumatic level throughout shock. In still other instances, the level of blood pressure attained following adrenalin injection decreased gradually and to a moderate extent in the course of shock (fig. 2). There was also a definite tendency for fluctuations in blood pressure to be accompanied by fluctuations in the level of adrenalin elevation in the same direction (fig. 2).

DISCUSSION. Cannon (3) proposed that hyperactivity of the sympathetico-adrenal system excited by sensory nerve impulses is an important factor in traumatic shock. This theory was based in part on the well known clinical observation that cold, pain and fear, all of which have been

shown to stimulate the sympathetic nervous system, tend to intensify shock following trauma.   It was also based on experiments which indicated that hyperadrenalinemia or continuous strong stimulation of the sympathetic system was capable of decreasing blood volume and lowering blood pressure significantly.   It was assumed that prolonged, marked arteriolar constriction causes shock as a result of fluid loss on the basis of capillary anoxia and consequent increased permeability.

While earlier experimenters had succeeded in producing fatal shock by injections of this hormone, they used massive doses beyond the physiological range.   For example, Erlanger and Gasser (8) working on dogs, injected 6 to 11 mgm. of adrenalin in 21 to 29 minutes and repeated this several times.   This greatly exceeds the 0.0032 to 0.0037 mgm. per kilogram per minute reported by Cannon and Rapport (9) to be the maximum physiological secretion of adrenalin in response to afferent stimulation.

Freeman (2) studied the effects of continuous injection of physiological amounts of adrenalin on blood volume and blood pressure.   Continuous intravenous administration of 0.001 to 0.006 mgm. per kilogram per minute of adrenalin to cats under dial over a period of two hours resulted in a rise in blood pressure at first from 140 to 210 mm. Hg followed by a fall to 180 mm. Hg.   After stopping the injection, the arterial pressure fell to 100 mm. Hg.   The average decrease in blood volume, measured by means of brilliant vital red in nine cats subjected to continuous intravenous adrenalin was 14 per cent.   On the other hand, after ergotoxine, adrenalin injection no longer affected the blood volume.

These experiments do not constitute convincing evidence that hyperadrenalinemia may play a rôle in the production of traumatic shock.   The decrease of blood volume reported is quite small.   Moreover, it must be pointed out that during the injection of adrenalin the blood pressure remained higher than normal and only fell, and to a moderate extent, following withdrawal of the hormone.   Also, results of a similar investigation by Hamlin and Gregersen (10) are contradictory to those of Freeman. Continuous injection of adrenalin in unanesthetized cats increased the blood volume.   Furthermore, nembutal anesthesia resulted in a 10 per cent increase of blood volume.   While adrenalin administration to cats under nembutal produced a decrease in blood volume, the volume did not fall below the pre-anesthetic level.   In an ingenious experiment, Prohaska, Harms and Dragstedt (11) failed to produce shock by continuous intravenous injection of physiological quantities of adrenalin for as long as two weeks in unanesthetized dogs.

There remains the evidence presented by Freeman (2) that physiological hyperactivity of the sympathetico-adrenal system results in a significant decrease of blood volume and blood pressure.   Hyperactivity of the sympathetic nervous system was produced by acute decortication of cats, resulting in "sham rage".   In eight experiments, the blood pressure fell

from 137 to 55 mm. Hg in 90 minutes. The average decrease of blood volume in fifteen experiments was 21.9 per cent. This fall of blood pressure and blood volume in the pseudo-affective state was prevented by preliminary sympathectomy or administration of ergotoxine.

Our own experiments on the pressor effects of adrenalin in traumatic shock provide no support to the sympathetic hyperactivity theory. One would expect that if a persistent marked vasoconstriction were present during shock, the influence of adrenalin on the blood pressure would be very greatly reduced or absent. On the contrary, during almost the entire post-traumatic period, the rise of blood pressure resulting from adrenalin was greater than normal. The view that sympathetic hyperactivity is not a feature of shock produced primarily by afferent nerve impulses (except terminally) is also supported by the observation that the nictitating membranes were quite relaxed and the pupils of normal diameter during the course of shock. This does not rule out the possibility that sympathetic hyperactivity may play a rôle in shock following hemorrhage or fluid loss (12, 13).

SUMMARY

1. Trauma to a hind limb in which local fluid loss was minimized resulted in fatal shock in $2\frac{1}{2}$ hours.

2. Shock was effectively prevented in such experiments by preliminary transection of the upper lumbar spinal cord.

3. Adrenalin produced a greater rise in blood pressure during shock than before trauma, except just before death, when the response of blood pressure to adrenalin suddenly disappeared.

4. No evidence was found to support the theory that hyperactivity of the sympathetic nervous system is responsible for the type of shock resulting primarily from afferent nerve impulses.

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AN ANALYSIS OF THE EXCITATORY AND INHIBITORY EFFECTS OF SYMPATHETIC NERVE IMPULSES AND ADRENALINE ON VISCERAL SMOOTH MUSCLE

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Stimulation of sympathetic nerves diminishes or stops the spontaneous movements of some visceral organs. For an understanding of this inhibitory effect it is important to know the changes in the functional state of visceral muscles produced by the nerve impulses. Experiments relating to this question, performed mainly on uterine muscle from the cat, will be reported in this paper.

A complication was introduced into these studies by the observation that the responses of the uterus to nerve stimulation were often diphasic, the inhibition being preceded by an excitatory action. Such responses have occasionally been observed before, but a more careful analysis suggested that they are the general type of response of the uterus to nerve stimulation.

No particular significance has hitherto been attached to such diphasic responses because it was accepted that the hypogastric nerve contains motor as well as inhibitory fibers. Various observations made it improbable, however, that the diphasic responses are due to specific excitatory and inhibitory fibers in the extrinsic nerves of the viscera. An attempt will be made in this paper to explain the experimental facts on the assumption that excitation and inhibition are two phases of the action of the same nerve impulses and that the character of the muscular responses, the preponderance of any one of these phases, is determined by the functional condition of the muscle.

METHODS. Cats under nembutal anesthesia were used in most experiments. Because uterine muscle is excitable electrically only during estrus (1), the animals received injections of theelin (500 int. units in oil at intervals of two days) for a few days before the experiment. One uterine horn was freed from the surrounding tissue and attached to an isometric lever. A firm ligature was tied around the other horn close to the body of the uterus. For the direct stimulation of the uterus, condenser discharges (7 μ F) were used. Cotton wicks soaked in Ringer's solution served as stimulating electrodes.

Uterine and intestinal strips were prepared as described previously (1).

The contractions of the small intestine of the cat and dog were recorded by a balloon and mercury manometer.

RESULTS. Theoretically it would seem possible that inhibition of an automatically contracting organ can be produced by two changes within the muscle: 1, an interference with the mechanism responsible for the initiation of impulses; 2, a decrease in excitability, blocking muscular conduction. These possibilities will be tested in the experiments described below.

A. *Excitability.* In the non-pregnant cat, stimulation of the hypogastric nerve, or adrenaline injected intravenously, completely inhibits the motility of the uterus (cf. 10, 17). During inhibition the electric excitability as determined by the threshold for single electric shocks was
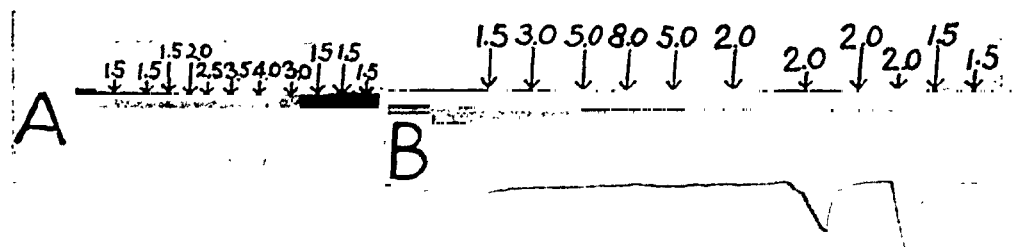
Fig. 1. Motility of the cat's uterus recorded by an isometric lever. During contraction, downward movement of the lever. Signal: stimulation of the hypogastric nerve (frequency about 20 per sec.). The excitability of the uterus was tested by stimulating the muscle directly with single condenser discharges (indicated by arrows). The numbers on the arrows are the voltages applied. Long arrows indicate ineffective stimuli. In B, the uterus became completely non-excitable during inhibition, in A weak responses were still elicited by strong stimuli. The experiments show a gradual rise in excitability following the cessation of nerve stimulation.

diminished or completely abolished (25 observations on 8 cats, fig. 1, A, B). Also the excitability for mechanical stimuli, tested by tapping the uterus with a blunt instrument, disappeared during stimulation of the hypogastric nerve.

These observations were confirmed in experiments on isolated uterine muscle. By the application of a few drops of adrenaline 1:500,000 the excitability of uterine strips, taken from animals in incomplete estrus (theelin injections for 4 days), was completely abolished and returned to normal within about 10 minutes. If the strips were taken from animals in full estrus (obtained by injections of theelin for 5 days) excitability was also diminished by adrenaline, but not completely abolished.

Similar results were obtained for intestinal muscle. Strips of the rabbit's small intestine which had been mounted in a moist chamber one-half to

one hour previously, were used. Such preparations, after they had become quiescent, responded to electric shocks by a contraction conducted over the entire muscle (2). Adrenaline $1:10^6$ raised the threshold 3 to 4 times and blocked conduction. Strong electric shocks still elicited local responses near the cathode.

In the isolated ureter of the cat and guinea pig, on the other hand, adrenaline increased electric excitability. Confirming previous observations (9), adrenaline also speeded up the conduction of peristaltic waves and often induced contractions.

In uterine muscle the effect of adrenaline varies in different species and under different physiological conditions. During estrus, adrenaline raised the threshold in guinea pigs only in about half the animals tested. The preparations never became non-excitable and in some there was no detectable effect. In two out of the ten animals tested, adrenaline produced a weak contraction. In the rabbit and in the pregnant cat a strong contraction was always produced under the same condition. These species differences run closely parallel with the well known differences in the responses to sympathetic nerve impulses (cf. 10, 16).

It becomes evident from these facts that adrenaline imitates both the excitatory and inhibitory effects of sympathetic nerve impulses. Observations which will be reported below confirm this conclusion.

B. *Diphasic responses.* In the cat's uterus a weak contraction preceding inhibition was previously found during the early stages of pregnancy (12) and rarely also in non-pregnant animals (5, 19). It was thought that in the non-pregnant cats in which this type of response was observed, the uterus had not completely returned to the resting state following a pregnancy, but no anatomical evidence for this assumption was found.

However the following observations (16 cats) show that, under suitable conditions, diphasic responses of the uterus can be obtained in every non-pregnant cat. During estrus, induced by injections of theelin, the movements of the uterus often, particularly in large animals, consist of powerful contractions separated by long intervals of rest. Stimulation of the hypogastric nerve, then, always elicited a strong contraction, followed by complete relaxation during which the muscle was unresponsive to further nerve stimulation (fig. 2A).

In smaller animals the uterus usually was in a state of tonic contraction on which only slight rhythmic contractions were superimposed. In this case stimulation of the hypogastric nerve always produced an initial contraction (fig. 2B).

The magnitude of the initial excitatory effect depends largely on body temperature. At normal temperature the effect sometimes could not be demonstrated clearly, but it was always observed, and markedly increased, after the body temperature was lowered to 33° to 36°. In some cases the

excitatory effect was the most prominent part of the response and inhibition followed only after prolonged stimulation.

The initial contraction occurred without any noticeable delay and direct observations showed that it began simultaneously with a blanching of the organ. This fact excludes the possibility that the contraction resulted from asphyxia due to vasoconstriction.

Diphasic responses to stimulation of the hypogastric nerve have been described also in other species. It was found by Cushny (4) in the rabbit,
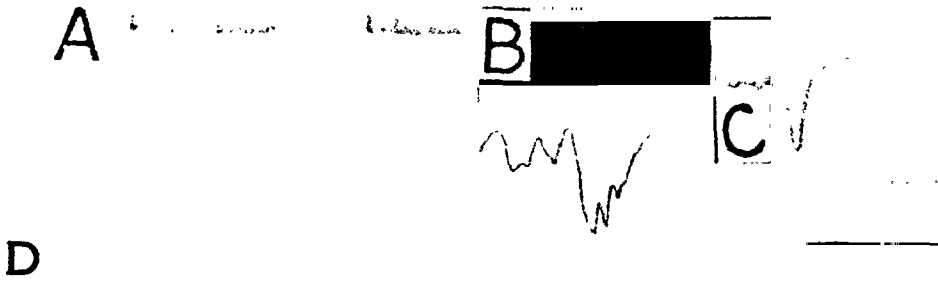
Fig. 2. Contractions of the cat's uterus in situ. The records show the initial contraction due to stimulation of the hypogastric nerve (A and B) and adrenaline (C and D).

A, uterus fully relaxed. The first upward movement of the lever is due to the contraction of the urinary bladder.

B, weak rhythmic contractions are increased by nerve stimulation (time intervals 6 sec.).

C, two injections of adrenaline (20 γ), time of injection indicated by signal.

D, responses to adrenaline (20 γ), time of injection indicated by upper signal. Time intervals in seconds. Because of the strong spontaneous contractions responses to single injections of adrenaline could not be demonstrated with certainty, but a second injection gave a contraction before a spontaneous contraction would have occurred.

and by Langley (13) in the dog (2 animals). These observations and the experiments just reported on the non-pregnant cat, where a purely inhibitory action of the sympathetic had been accepted, make it probable that diphasic responses occur more frequently than generally assumed. Therefore, contraction followed by inhibition may be considered as the general type of response of the uterus to sympathetic nerve impulses. The well known variations in the effects of nerve stimulation thus appear merely as quantitative differences in the magnitude of the excitatory and inhibitory phase of the response.

The diphasic responses are generally (cf. 10, 17) explained on the assumption that the hypogastric nerve contains excitatory and inhibitory nerve fibers. On the basis of the theory of chemical transmission of nerve impulses it appears logical to assume that these antagonistic nerve fibers are adrenergic and cholinergic (cf. 17). However atropine, even in concentrations many times greater than necessary for blocking the vagus, did not change the responses of the cat's uterus (in confirmation of Kennard's observations (12) on the pregnant uterus). Furthermore it is well known that in animals where sympathetic nerve impulses have an excitatory action also adrenaline increases motility. It is, therefore, probable that the excitatory as well as the inhibitory action of sympathetic nerve impulses is due to adrenergic fibers.

It is particularly significant that adrenaline alone, injected intravenously, can elicit diphasic responses. In every instance the action of this drug, whether it was purely inhibitory or diphasic, agreed closely with the effects produced by stimulating the hypogastric nerve (fig. 2). These facts do not exclude the possibility of a supply of cholinergic fibers to the uterus, but such fibers, if they exist, do not determine significantly the nature of the responses.

In agreement with the results of Kennard (12) on the pregnant uterus, the inhibitory phase often, but not always, was more prominent after injection of adrenaline than after nerve stimulation. This difference may be explained by the different rate at which the concentration of adrenaline rises in the muscle and does not indicate an essential difference in the responses. On the basis of the theory which will be presented below it may be expected that the initial contraction is the smaller the more rapidly the concentration of adrenaline rises within the musculature.

Diphasic responses produced by adrenaline have also been observed in the dog (20) and monkey (11).

To reconcile the fact that adrenergic nerve fibers, or even adrenaline alone, can produce in the same muscle excitation as well as inhibition, with the theory of specific excitatory and inhibitory nerve fibers, it becomes necessary to suppose that the antagonistic effects occur at different regions of the muscle. Kennard assumed that the excitatory and inhibitory action is due to the formation of two different kinds of sympathin liberated in different regions of the muscle. However this hypothesis disagrees with the fact that on continuous stimulation the initial contraction is followed by complete inhibition and that the muscle remains unresponsive thereafter for one minute or longer (fig. 2).

In view of the apparent impossibility of separating the excitatory from the inhibitory effects of sympathetic nerve impulses it seems worth while to consider the possibility that these effects represent two phases of the action of the same nerve impulses.

As stated above, inhibition may conceivably be brought about by one

or both of two changes: 1, interference with the processes responsible for the initiation of the impulses, like the depression of the pacemaker of the heart; 2, a decrease in excitability resulting in a block for muscular conduction, as demonstrated for cardiac and visceral smooth muscle.

Experimental evidence indicates that these two changes are essentially independent from one another. In the heart it has been shown that the initiation of beats is not determined by the level of electric excitability of the pacemaker (6) and, consequently, the slowing of the beat produced by vagal impulses cannot be considered as an effect secondary to the decrease in excitability. Furthermore it has been found that acetylcholine, although lowering the excitability of cardiac muscle (cf. 8) has only an inotropic and no chronotropic action on the pacemaker if applied in low concentration (7), showing that these two changes are separate effects of vagal stimulation.

Similar evidence was obtained for intestinal muscle. Adrenaline, although it decreases the excitability of the muscle, never diminishes the
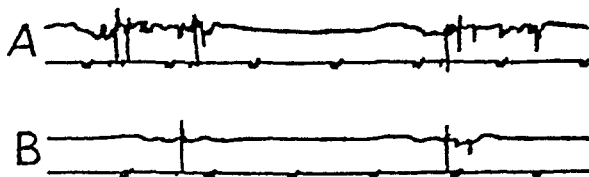
Fig. 3. Action potentials from the small intestine of the rabbit in situ. A, normal pendular movements: B, after intravenous injection of 15 γ adrenaline.

frequency of the movements, provided that the concentration of the drug is not high enough to stop the movements entirely.

Adrenaline diminishes the number of impulses discharged during each contraction (fig. 3). This effect is responsible for the decrease in the amplitude of the movements. It can be explained by the lowering of the average level of excitability, which cuts short each burst of impulses sooner than would normally occur.

The diphasic responses can be explained as follows. On the basis of the observations just reported it appears reasonable to assume that sympathetic impulses tend to initiate muscular impulses and that, at the same time, they lower excitability. It would be expected, then, that nerve impulses or adrenaline first increase activity. During continuous stimulation, however, the discharge of the muscle will be stopped by a block of muscular conduction as soon as the excitability has dropped below a certain level. Thus a diphasic response will result. If, on the other hand, the block is established rapidly the excitatory phase may be masked. Finally a purely excitatory action will be found if the excitability is not appreciably depressed by the sympathetic nerve impulses, as has been found under certain conditions (p. 629).

This theory explains the diversity of the responses of the uterus as the result of quantitative differences in the functional condition of the muscles. A reversal in the action of sympathetic impulses and adrenaline in some species, then, may be considered as an expression of the change in the functional state of the uterine musculature as it occurs during pregnancy and estrus, and it becomes unnecessary to postulate alterations in the function of the nerve fibers.

In conclusion it may be said that the hypothesis of specific motor and inhibitory sympathetic nerve fibers to the uterus has not contributed to the understanding of the diversified responses of this organ. This hypothesis seems on a rather insecure basis because purely excitatory and inhibitory effects of nerve stimulation are rather the exception than the rule, and it cannot account for the fact that excitatory as well as inhibitory effects are produced in the same organ by adrenaline alone or by adrenergic nerve fibers. It appears, therefore, that the assumption of a dual action of sympathetic nerve impulses offers a simpler explanation of the responses of the uterus than the hypothesis of a dual sympathetic innervation.

Also in other visceral organs it has seemed necessary to postulate the presence of excitatory and inhibitory sympathetic nerve fibers. In the urinary bladder (cf. 10, 15), adrenaline and stimulation of the hypogastric nerve produce a diphasic response like that of the uterus. In the gastrointestinal tract the sympathetic is predominantly inhibitory but excitatory effects are often obtained, particularly in the stomach (cf. 14). The conclusions regarding the nervous control of uterine motility may possibly be applied also to these observations, particularly to cases where adrenaline alone has a diphasic effect.

Several older investigators (cf. 10) reported an increase in the motility of the small intestine as a response to splanchnic stimulation. These observations were confirmed in experiments on 4 cats and 2 dogs. Contractions could be induced in the quiescent intestine by splanchnic stimulation, but these responses occurred only at very low body temperatures (33° to 30°) and they always had a long latent period (15 to 20 sec.). It seemed possible, therefore, that the contractions resulted from the asphyxia produced by vasoconstriction and, in fact, asphyxia produced by occluding the aorta also increased motility in these animals. It seems probable, therefore, that the sympathetic has a purely inhibitory action on the musculature of the small intestine.

SUMMARY

Sympathetic nerve impulses and adrenaline lower or abolish the excitability of the non-pregnant cat's uterus. Adrenaline was shown to produce this effect also on intestinal strips.

In the non-pregnant cat's uterus the inhibition brought about by stimu-

lating the hypogastric nerve is always preceded by an excitatory effect. This finding and occasional observations on other species suggest that diphasic responses are the general type of response of the uterus to nerve stimulation. The magnitude of each one of the two phases of the response varies greatly under different conditions and in different species.

Both the excitatory and inhibitory effects are produced by adrenergic nerve fibers as shown by the fact that adrenaline alone gives essentially the same response as sympathetic nerve impulses.

These results cannot readily be explained on the assumption of specific excitatory and inhibitory nerve fibers. It is suggested that excitation and inhibition represent two phases of the action of the same nerve impulses. Specifically, the observations are explained by assuming that inhibition is due to the diminution in excitability, which leads to a block of muscular conduction, and by the further assumption that the sympathetic nerve impulses also tend to set up muscular impulses. The excitatory action will be suppressed as soon as the level of excitability has dropped sufficiently to block muscular conduction. A diphasic response will result usually but one of the phases may also be masked by the other. These assumptions explain the great variety of responses of the uterus and the reversal of the responses during pregnancy or estrus, as the result of the experimentally demonstrated quantitative differences in the properties of the muscles without assuming any changes in the nervous mechanism.

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THE MEASUREMENT OF VENOUS PRESSURE IN MAN ELIMINATING THE HYDROSTATIC FACTOR

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The pressure at any point in a vein may be considered to be the result of the following factors: the residuum of the total head of pressure created by the heart beat which is transmitted through the capillaries to the vein, the resistance to the flow of blood from the point in the vein where the pressure is measured to the right auricle, the pressure in the right auricle, and the hydrostatic pressure exerted by the column of blood extending from the point in the vein where the pressure is measured to the point in the right auricle where the blood leaves the right auricle.

Most workers, in order to eliminate the hydrostatic factor, have taken the level of the right auricle as the point to which venous pressure should be referred. However, Clark, Hooker, and Weed (1934) have shown that in the dog the reference point, as they use it, is not the right auricle but consists of two points, one located several centimeters cephalad to the heart for the head section of the animal, and another located several centimeters caudad to the heart for the tail section of the animal. However, since blood leaves the venous system at the right auricle, and so long as there is a column of blood extending from the right auricle to the point in the vein where venous pressure is measured, there is a hydrostatic pressure equal to the height of this column of blood. In order to eliminate this hydrostatic pressure from the venous pressure measurement, it is necessary to refer the venous pressure to the level of the right auricle.

There is little agreement as to the position of the right auricle in the chest. The position of the right auricle in the supine subject has been taken to be at the following points by different workers: one-half the distance through the chest in the anterior-posterior line drawn from the subcostal angle (von Recklinghausen, 1906); five centimeters dorsal to the sternum at the fourth costal cartilage (Moritz and Tabora, 1910); at the junction of the anterior third and the middle third of the anterior-posterior line drawn from the sternum at the fourth intercostal space (Eyster, 1929); and ten centimeters ventral to the level of the skin of the back (Lyons, Kennedy and Burwell, 1938). These reference points differ widely and

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in some cases the extreme points differ by as much as ten centimeters; in most cases they differ by about four centimeters.   This causes the venous pressure, as measured, to differ by this much.

It has been attempted here to determine more accurately the position of the reference point and to measure the venous pressure by a procedure that eliminates the hydrostatic factor in the measurement.   If the venous pressure in the antecubital vein of a subject in the supine position is measured by the direct method, this pressure is composed of the hydrostatic pressure exerted by the column of blood extending from the point in the vein where the pressure is measured to the right auricle plus the remaining venous pressure which has no hydrostatic component (fig. 1a). If the subject is turned to the prone position and the pressure measured in a similar manner, this pressure is composed of a hydrostatic pressure
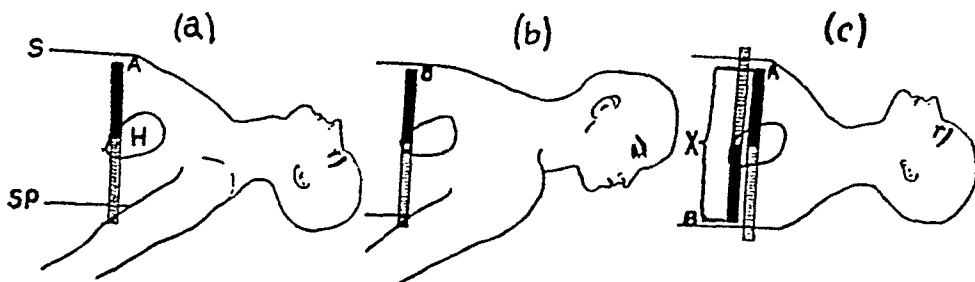
Fig. 1. Diagrams showing the height of the column of saline (venous pressure) in the supine and prone positions.   Solid black area represents the venous pressure having no hydrostatic component.   Shaded area represents the hydrostatic component.   (a), supine.   (b), prone.   (c), composite picture of the two positions. A, level of the top of the saline column with the subject in the supine position.   B, level of the top of the saline column with the subject in the prone position.   H, heart.   S, sternum.   SP, spine.   X, the sum of the two venous pressures with the hydrostatic pressure eliminated.

component equal to the height of the column of blood extending from the point in the vein where the pressure is measured to the right auricle plus the remaining pressure which has no hydrostatic component (fig. 1b). In the above two pressure measurements the position of the right auricle is not known, but the pressures can be referred to the level of the skin lying over the spine, at the level of the fourth intercostal space, which is a fixed point.   If a composite picture (fig. 1c) is made of the two above measurements, and it is assumed that the position of the right auricle relative to the spine does not change when the subject changes from the supine to the prone position, then it is seen that the distance between the points A and B (fig. 1c) represents the sum of two venous pressure measurements, and that the hydrostatic pressures have been eliminated from the venous pressure measurement.   If the venous pressure (less the hydrostatic component) does not change as a result of the change from the supine

to the prone position, then the mid-point between A and B (fig. 1c) represents the reference point in the right auricle.

Thus, if the venous pressure is measured with the subject in the supine position and then measured with the subject in the prone position, using the spine as the reference (zero) point in both cases, the sum of these two venous pressure measurements divided by two equals the venous pressure with the hydrostatic component eliminated; and the reference point is located at the point in the chest midway between the tops of the two columns of saline in the two pressure measurements, as shown in figure 1c.

It should be noted in determining venous pressure by this method that in the supine position when the top of the column of saline is ventral to the spine the pressure in the vein, using the spine as the reference (zero) point, is positive. When the subject is in the prone position and the top of the column of saline is ventral to the spine, the pressure in the vein, using the spine as the reference (zero) point, is negative.

METHOD. Venous pressure was measured in the antecubital vein in ten normal subjects in the supine position by a modification of the direct method of Moritz and Tabora (1910) using physiological saline in place of citrate in the manometer system. The subject lay on a flat table with a meter stick of 0.8 cm. thickness lying between the spine and the table in order to insure accurate location of the position of the spine. The thickness of the chest in the anterior-posterior diameter at the fourth intercostal space was measured. After the subject had been relaxed on the table for between ten and fifteen minutes with the extended arm lying on a smooth board posterior to the level of the spine and abducted to approximately forty-five degrees, the needle was inserted, taped in place and a venous pressure reading taken referring the pressure to the level of the spine as zero. The arm was raised a few centimeters by elevating the board on which the arm rested and then another pressure reading was taken. This was continued until the point in the vein where the pressure was measured was at the level of the sternum or above. The subject was then turned to the prone position, the needle remaining in place, and the arm extended, abducted to about forty-five degrees, and placed on a smooth board well below the sternum. The venous pressure was determined and then the arm elevated a few centimeters and another pressure reading was taken. This was continued until the point in the vein where the pressure was measured was approximately at the level of the spine. The level of the spine was determined and all pressure measurements referred to the spine as zero. In this way the venous pressure was determined in each subject using all of the reference points described above.

RESULTS. With the subject in the supine position venous pressure was found to vary widely in each subject depending on the reference point used (table 1). Lower values for venous pressure were obtained using the refer-

ence point of Moritz and Tabora and that of Eyster than were obtained using the other reference points. Venous pressure values obtained using the reference point of von Recklinghausen were close to the values obtained using the method described here. The venous pressure values obtained using the reference point of Lyons et al. were close to the values obtained using the method described here in all cases except that of a very thick chested individual in which case the value obtained using the reference point of Lyons et al. was higher than that obtained by any other method.

TABLE 1

S	CHEST DIAM-ETER	VENOUS PRESSURE MEASURED IN CENTIMETERS OF SALINE								X	R. P.
		M. and T.		E.		v. R.		L. et al.			
		Supine	Prone	Supine	Prone	Supine	Prone	Supine	Prone	Supine and prone	
	cm.										
1	19.4	8.5	19.8	10.0	18.3	13.2	15.1	12.9	15.4	14.1	8.7
2	18.3	6.7	16.2	7.8	15.1	10.9	12.0	10.0	12.9	11.4	8.5
3	19.5	7.1	17.1	8.6	15.6	11.9	13.7	11.6	12.6	12.1	9.5
4	20.0	6.0	10.8	6.7	9.8	9.9	8.0	9.9	5.8	7.8	12.0
5	25.7	4.6	17.4	8.1	14.4	12.5	11.7	15.3	10.8	11.2	14.0
6	18.5	5.3	16.3	5.8	15.3	8.9	13.2	8.1	10.6	9.3	8.8
7	19.8	8.0	12.3	8.0	10.7	8.5	8.9	8.3	9.0	7.9	10.4
8	17.8	6.0	12.4	6.1	11.5	8.0	9.1	6.9	10.2	8.3	8.7
9	18.4	5.3	11.6	6.4	10.5	9.5	7.4	8.7	8.2	8.4	10.2
10	18.9	8.9	19.1	10.2	17.8	13.4	14.6	12.8	15.2	14.0	8.8
Av.....	19.6	6.6	15.3	8.0	13.9	10.7	11.4	10.4	11.1	10.4	10.0

Summary of data on venous pressure determinations in the supine and prone positions using different reference points. S, subject; M. and T., reference point of Moritz and Tabora; E., reference point of Eyster; v. R., reference point of von Recklinghausen; L. et al., reference point of Lyons et al.; X, using the method described here; R. P., reference point, as determined by the method described here, expressed in centimeters ventral to the spine; Av., average.

Although the reference points of von Recklinghausen, Moritz and Tabora, Eyster, and of Lyons et al. were not intended to be used in the determination of venous pressure with the subject in the prone position, the venous pressure in the prone position was measured using these reference points in order to show the wide variation of venous pressure depending on the reference point used (table 1).

The venous pressure measured with the subject in the prone position using the reference point of Moritz and Tabora and that of Eyster was high compared to the values obtained by using the other methods, while the opposite was the case with the subject in the supine position (table 1).

The normal venous pressure was found to vary between 7.8 and 14.1 cm. of saline with 80 per cent of the cases having a pressure between 7.8 and 12.1 cm. of saline using the method described here.

Using the spine as the reference (zero) point for the venous pressure measurement, it was found that raising or lowering the vein caused no change in the venous pressure so long as the vein remained below the middle region of the chest. Elevation of the vein above the middle region of the chest caused an elevation in the venous pressure (fig. 2). This was seen both in the supine and prone positions, and similar results were obtained on models using thin walled collapsible rubber tubes to represent veins.

DISCUSSION. There is a possibility that in determining venous pressure by the method described here the shift from the supine to the prone position changes the venous pressure and the position of the right auricle, and thus the venous pressure, as measured, is inaccurate and the location of the reference point is inaccurate. That this is not the case is suggested by the following observations. Hooker (1914), White (1924), Doupe et al. (1938), and others have shown that vasodilatation or vasoconstriction in the arm causes little or no change in venous pressure, thus it is unlikely that the shift from the supine to the prone position causes any change in the pressure transmitted through the capillaries to the vein. Also, so long as the subject is relaxed and there is no constriction between the point where the venous pressure is measured and the heart, the resistance to flow to the heart is probably not changed by the change from the supine to the prone position. There is the possibility, however, that the pressure in the right auricle is changed as a result of the change from the supine to the prone position. Although there are no data on this point in man, intra-auricular pressure measurements in three anesthetized dogs, with the chests closed, showed that the pressure either did not change or was decreased by one centimeter of saline pressure when the animal was turned from the supine to the prone position. This suggests that there is little or no change in right auricular pressure in man due to the change from the supine to the prone position. Since the base of the heart is firmly fixed posteriorly, it is unlikely that the shift from the supine to the prone position causes any marked change in the position of the right auricle relative to the spine. However, if the position of the right auricle is changed due to the shift from the supine to the prone position, the reference point, as determined by the method described here, will lie at a point midway between the two positions of the right auricle (fig. 1c). Also, the venous pressure will be decreased, assuming that the heart falls forward in the prone position, by an amount equal to the pressure exerted by one-half the height of the column of blood extending between the two positions of the right auricle.

Thus it seems unlikely that the shift from the supine to the prone posi-

tion causes any change in the venous pressure. However, if the venous pressure does change due to the shift from the supine to the prone position then the value obtained for venous pressure using the method described here will be the average value of the venous pressure in the two positions with the hydrostatic factor eliminated.

The rise in venous pressure that was found when the arm was placed above the heart level confirms the observations of Carrier and Rehberg (1923) and those of Lyons et al. (1938). These investigators attributed this rise to the elevation of the vessels above heart level causing the
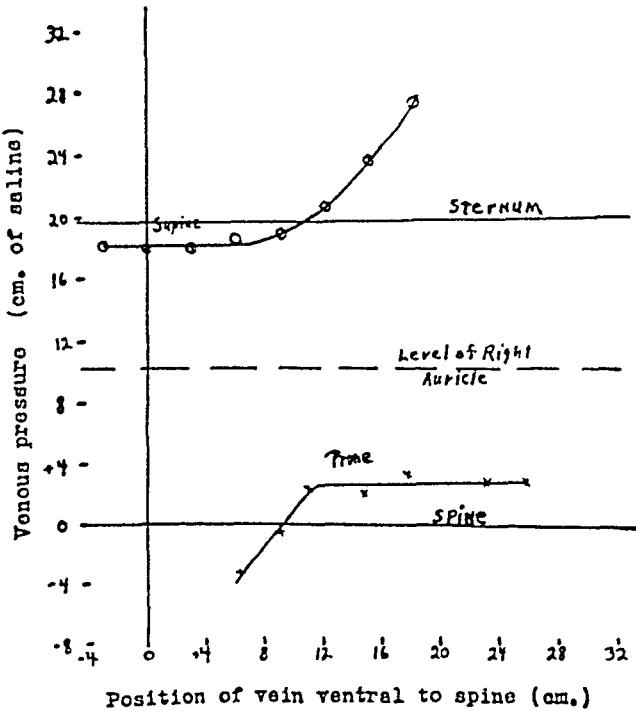
Fig. 2. Graph showing the height of the top of the column of saline (venous pressure) ventral to the spine with the arm in different positions relative to the spine. +, ventral to spine. −, dorsal to spine. The level of the right auricle was determined as described in the text.

vessels to collapse and thereby increasing the resistance to flow between the point where the pressure was measured and the heart, and thus increasing the venous pressure. However, there is the possibility that this rise in venous pressure is the result of constriction of the vein caused by angulation at the shoulder when the vein was above heart level. That this is probably not the case is shown by the fact that a rise in venous pressure was found with the subject in the prone position only when the vein was above heart level (fig. 2). Since angulation of the arm at the shoulder was the same with the vein above heart level in the prone position as it was with the vein below the heart level in the supine position

and since the rise in venous pressure was seen only when the vein was above the heart level, the angulation must have played no part in the pressure rise.

SUMMARY

Venous pressure was determined in the supine and prone positions in ten normal subjects using a modification of the direct method of Moritz and Tabora. Venous pressure values obtained using the reference point of Moritz and Tabora and that of Eyster were markedly different from the values obtained using the reference points of von Recklinghausen, Lyons et al., and the method described here. The reference point of Moritz and Tabora and that of Eyster appear to be placed too far ventrally.

It is suggested that venous pressure be determined in the following manner in order to eliminate the hydrostatic factor. The pressure in the antecubital vein is measured by the direct method with the subject in the supine position, the arm lying well below the center of the body and abducted to approximately 45 degrees. The subject is turned over into the prone position and the pressure measured again with the arm well below the center of the body and abducted to about 45 degrees. All pressures are referred to the level of the spine as zero. The sum of the two pressures divided by two equals the venous pressure, and the reference point is located at the point in the chest mid-way between the tops of the two columns of saline in the two pressure measurements as shown in figure 2.

Venous pressure determined in this manner varied between 7.8 and 14.1 cm. of saline with 80 per cent of the cases varying between 7.8 and 12.1 cm. of saline.

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RECIPROCAL INNERVATION IN THE SMALL INTESTINE

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The small intestine is supplied by two efferent nerves which affect its motility—the vagi and the splanchnics. Most investigators are in general agreement that the former are motor, the latter inhibitory (for a review of the evidence on this point see Hukuhara, 1932). The fact that an effector is supplied by two nerves, which on peripheral excitation produce opposite actions, does not necessarily mean that the organ is reciprocally innervated. It does, however, immediately suggest the possibility that this type of coördination is present. The experiments here described were designed to test whether reciprocal innervation does indeed exist in the gut. The method consisted of destroying either the motor or inhibitory nerves and observing whether augmentor or depressant reflexes are still obtainable. If, when humoral agencies are excluded augmentor responses can be elicited in the absence of the motor nerves, or if inhibition is apparent after destruction of the depressant fibers, then—since only two extrinsic nervous sources are known to act on the gut—the altered motility must be mediated by a diminished activity of the remaining nerves. The persistence of such reflexes, then, would prove reciprocal control of an autonomic effector.

METHODS. Most of the experiments were performed on young vigorous cats, but in a few cases rabbits were used as test animals. In order to insure the presence of lively intestinal movements the following expedients were employed. 1. Sometimes a dose of castor oil was administered 14 to 24 hours before the experiment. 2. In all cases (regardless of whether or not a purgative had been given the day before) the animals were fed a meal of salmon and milk 2 to 3 hours before the start of the experiment.

The cats were anesthetized with urethane, 1.25 grams per kgm. in the femoral vein, or dial (Ciba) 0.65 to 0.75 cc. per kgm. intraperitoneally (slightly smaller doses were employed for rabbits). The animal was then placed on a warming pad and its temperature was kept within the normal range.

After the insertion of a tracheal cannula the abdominal cavity was opened. The influence of the adrenals was routinely abolished by tying off each gland *en masse* with stout thread. When the experiment required

removal of the splanchnics, these nerves were identified as they emerged beneath the diaphragm on either side and 1 to 2 cm. were extirpated. In some instances the semilunar ganglion also was cut out. A small rubber balloon, made of condom rubber, or more usually fashioned from the finger of an ordinary surgical glove, was attached to a glass tube of narrow bore by means of rubber cement. It was passed into the lumen of the duodenum through a short longitudinal incision. A stitch carried through both layers of muscle and tied around the glass tube held the balloon in place. The balloon was connected by rubber tubing and a T-tube to a Marey capsule and a water manometer. The pressure in the system was raised to 5 to 10 cm. of water and the manometer was then disconnected by means of a stopcock. Thus a record of duodenal contractions and the tone of the musculature was obtained on a kymograph. The system was tested for leaks before each experiment. In order to obviate the disturbing influences of drying, cooling and handling, the contents of the peritoneal cavity were carefully wrapped in a towel soaked in warm Ringer's solution, while the above procedures were being carried out. The surgery was performed as rapidly as possible and with care to prevent unnecessary trauma. After the operations were completed, a small quantity of warm Ringer's was introduced into the abdominal cavity, the guts were carefully arranged to avoid kinking and the omentum was spread over them. Then the abdominal wall was closed with hemostats or large paper-clips, or sewn with linen thread.

The sciatic nerve on one side was bared, cut, and prepared for central stimulation. The vagi on both sides were isolated and made ready for section or central excitation. In all experiments the cervical sympathetic trunks were separated from the vagi and cut. Generally, if the vagi were to be excluded, they were cut before any records were taken. In some cases, however, it was thought worth while to investigate a possible tonic action of these nerves. Hence, they were not cut until the experiment was in progress and the intestinal movements were being recorded.

In a few of the earlier animals the guts were allowed to remain within the closed abdominal cavity while the experiments were being conducted. It was frequently found, however, that there was a tendency for the motility to become seriously impaired after 1 to 2 hours. Consequently, the later experiments were carried out with the intestines floating in a Ringer bath, the temperature of which was kept constant at 38°C. This method had the advantage of preserving the vigor of the movements for several hours. It also allowed the advantage of direct observation of the gut and avoided the complications which contractions of the abdominal musculature occasionally created.

Some animals were chronically sympathectomized or vagotomized 7 to 16 days before the acute experiment. Bilateral abdominal sympathec-

tomy was performed aseptically according to the technique of Cannon, Newton, Bright, Menkin and Moore (1929). In addition, 1 to 2 cm. of the splanchnics were removed on both sides and the celiac ganglion was extirpated. Parasympathetic fibers to the small intestine were destroyed by stripping the vagal filaments from the anterior and posterior surface of the esophagus immediately after it pierced the diaphragm.

Shielded silver-wire electrodes were used for stimulation of the nerves.

A Harvard inductorium with 1.5 to 3.0 volts in the primary circuit and varying positions of the secondary coil provided faradic induction shocks.

RESULTS. A. *Tonic action of the vagi.* Most investigators have concluded that the vagi are either entirely or primarily motor for the small intestine. This conclusion led to a study of the effects of section of these nerves to learn whether or not they exhibited a tonic action on intestinal motility. Bayliss and Starling (1899) were unable to detect, in anesthetized dogs, the existence of tonic vagal influence on the intestines. Hotz (1909) and L. R. Müller (1931) subscribed to the view of the English workers. The data of Aehle (1936) on a small series of rabbits are equivocal. Cannon (1906), in contrast to the foregoing investigators, assigned to the vagi a tonic action. He found, by means of x-ray studies carried out several days after cats had recovered from double vagotomy, that the passage of a meal of lean beef through the intestine was slower than normal. Also Alvarez and Mahoney (1924) stated that vagal section in rabbits reduced the tendency for "peristaltic rush" along the bowel.

In an attempt to add more data on the existence or non-existence of vagal tone, 8 cats were studied. After the intestinal movements were being recorded the vagi were cut in the neck. In 4 cats of this series the splanchnics had been previously sectioned, while in the remaining animals they were intact. In 5 experiments vagotomy resulted in a decrease in the rate and extent of the contractions. In 2 cases the results were not striking, and only a slight slowing of the movements occurred. In the other cat vagotomy was without effect on intestinal activity.

No statement can be made regarding the period of time during which effects referable to vagus section remained in the 7 animals. In some cases slowing or even complete inhibition appeared rapidly, and after a few minutes the rate approached the normal. The new rate, however, was always slower than before severing the vagi. Generally, the amplitude of contractions was first reduced and then gradually approached the normal in size. Figure 1 illustrates the most striking example of vagal tone. In the animal from which this record was obtained the splanchnics were cut, the adrenals were tied and the celiac ganglion was removed. Interruption of the vagal supply caused a prompt inhibition of all movements. Approximately 12 minutes later the intestines began to move rhythmically

again, although the contractions occurred less frequently and the individual beats developed less tension than before the vagi were cut.

B. *Reflex changes produced by sciatic nerve stimulation.* Excitation of an afferent nerve and various sensory stimuli, according to numerous observers, inhibit peristalsis and decrease intestinal tone. These observations were readily confirmed by faradizing the central stump of the sciatic nerve. Vagal section, either at the time of the experiment, or 1 to 2 weeks previously, did not abolish the reflex inhibition produced by sciatic stimulation.

Most investigators state that cutting the splanchnic nerves removes the possibility of obtaining reflex gastro-intestinal inhibition (Hotz, 1909; v. Lehmann, 1913; Morin and Vial, 1934). My results are in disagreement with this view. In the first experiments simple splanchnic section was performed and then the sciatic was stimulated. The presence of reflex inhibition led me to suspect—because of the contrary conclusions of earlier workers—that not all inhibitory fibers to the gut had been interrupted by splanchnicotomy; for it might be that a few fibers from the upper lumbar region ran, independently of the splanchnics, to the celiac ganglion. Consequently (in 8 cats) the celiac ganglion was removed when the splanchnics were cut. Figure 2 shows the typical inhibition after this possible source of error had been excluded.

In order to rule out any conceivable sympathetic supply and to obviate the possibility of humoral agencies (Youmans and Meek, 1937) 2 animals were subjected to bilateral abdominal sympathectomy and splanchnicotomy, removal of the celiac and superior mesenteric ganglia 9 and 16 days before the final acute experiment, at which time the adrenal glands were removed. These animals exhibited reflex intestinal inhibition, just as those in which less drastic surgery had been performed.

In the animals subjected to the different operations used to destroy possible sympathetic influences some variations in response occurred. Such were: latency, duration and extent of inhibition, and intensity and duration of the stimulus required to elicit the reflex. But the remarkable constancy of the inhibitory phenomena convinced me that they could be produced in the absence of all known depressant neural pathways. To prove that reflex inhibition was indeed mediated by the vagi—i.e., by a diminution in the normal vagal motor activity—these nerves were cut in the neck and the sciatic was again stimulated. Difficulties often arose in this phase of the experiment, for, frequently, when the vagi were destroyed the activity of the gut was permanently reduced (section A, p. 644) and the preparation was no longer so favorable for the study of inhibition. In some animals the gut remained fairly active after vagotomy. In such cases sciatic stimulation failed to evoke inhibition of the extrinsically denervated intestine.

Although a decrease in the rate and amplitude of the movements and a decline in the tone of the sympathectomized gut was the usual result of central sciatic stimulation, in 2 of 16 such experiments a marked *increase* in rhythm and tone occurred (fig. 3). The reason for these atypical results is obscure.

C. *Reflex changes induced by central vagal stimulation.* In view of the numerous reflex parasympathetic responses produced by central vagal stimulation this nerve was selected as one likely to produce augmentor intestinal effects. Conflicting reports concerning reflex vagal effects on the intestine can be discovered in the literature. Bunch (1897) at first stated that vagal stimulation was ineffective, but later (1899) considered

Fig. 1   Fig. 2   Fig. 3

Fig. 1. Intestinal movements of cat under urethane. Splanchnic nerves cut, adrenals tied, celiac ganglion removed. Signals represent section of vagi in neck. In this and subsequent records the lowest line records 30-second intervals.

Fig. 2. Cat, urethane. Splanchnic nerves cut, adrenals tied, celiac ganglion removed. At signals, stimulation of central end of left sciatic nerve, 1.5 volts, 6 cm.

Fig. 3. Atypical record obtained on sciatic nerve stimulation. Cat, urethane. Splanchnic nerves cut, adrenals tied, celiac ganglion removed. At signal, right sciatic nerve stimulated centrally, 1.5 volts, 6 cm.

that it occasionally caused increased activity of the longitudinal coat if the other vagus was intact. Bayliss and Starling (1899) always observed inhibition if the vagus on the opposite side and the splanchnics were intact, and no effect if the splanchnics were cut. v. Lehmann (1913) secured either increases or decreases of peristalsis in the dog when the splanchnics and one vagus remained.

Under the conditions of my experiments, when the splanchnics were intact, increased motility was always brought about by stimulating one vagus centrally, provided the opposite nerve was untouched. Figure 4 is representative of 5 experiments, in each of which augmentation of motility followed faradization of the vagus. Even when the guts were

more active than in the animal from which figure 4 was obtained, vagal stimulation elicited a definite, though not so marked increase of contractions.

When one intact vagus represented the sole extrinsic nerve supply central stimulation of the other vagus also produced heightened motility. This response was abolished when the remaining vagus was severed.

If both vagi were sectioned and only the splanchnics connected the gut with the central nervous system, stimulation of the central end of either vagus brought about varying results. In 2 animals no alteration of intestinal activity occurred. In 2 others the intestines were inhibited. In the remaining 4 cats evidence of reciprocal innervation was obtained (fig. 5). That is, if distant humoral effects may be excluded, the increased movements could have been induced only by inhibition of the normally
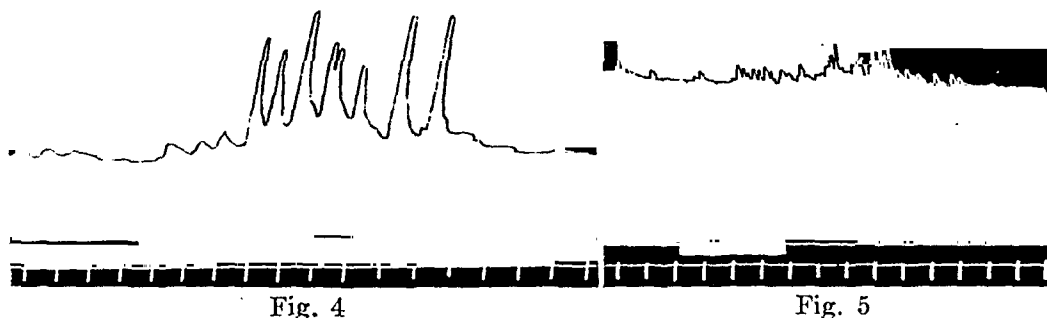
Fig. 4   Fig. 5

Fig. 4. Cat, urethane. Adrenals tied. Splanchnic nerves and one vagus intact. At signal, stimulation of central end of left vagus, 3 volts, 6 cm.

Fig. 5. Cat, urethane. Both vagi cut in neck. Splanchnics intact. At signal, right vagus stimulated centrally, 1.5 volts, 9 cm.

depressant influence of the splanchnics. The changes in motility were not due to chance variations in the intrinsic rhythmicity of the gut, since, after the splanchnics also were removed, neither inhibition nor augmentation took place when the vagus was stimulated. This operation, moreover, excluded the possibility of humoral depressant or excitant substances, released by nerves other than the splanchnics and acting upon the gut.

DISCUSSION. The experiments reported in section A show that the vagi exert a tonic motor influence on the movements of the intestinal tract, since the effect of destroying these nerves is to reduce the rate and amplitude of the contractions (fig. 1). The decline in motility after double vagotomy might be assigned wholly to the now-unchecked reign of tonic and reflex inhibitory activity of the splanchnic nerves. The vagi act, however, not merely to offset the splanchnics but are required for establish-

ment of a tonic state, because peristaltic depression occurs even when the vagal section follows splanchnic section.

The results of reflexly induced changes of intestinal motility cannot be adequately accounted for on the basis of complicating circulatory changes. In the first place, the evidence of Van Braam-Houckgeest (1874) and of Bayliss and Starling (1899) and others proves the independence of motility from local vascular changes. Also, from the present experiments it is apparent that reflex inhibition may occur with either a pressor (sciatic) or depressor (vagal) response. The objection might be raised that both these circulatory changes could be responsible for local asphyxia—the rise in pressure because of the constriction of the splanchnic arterioles, and the pressure fall by pooling of blood in the viscera and stagnation resulting from the decreased blood flow. On that basis, however, one could not explain the occasional enhanced motility seen on sciatic stimulation (fig. 3), nor the motor effects following central vagal excitation after unilateral or bilateral vagotomy (figs. 4 and 5).

Intestinal inactivity arising from sciatic stimulation when the sympathetic nerves are destroyed is not due to an outpouring of adrenaline, since the adrenal glands were excluded routinely. Nor is it likely that the reflex release of sympathin can account for the changes, because the latencies are in most cases too short to allow action of a distant humoral agent (fig. 2). The precautions described above (p. 645) to insure complete sympathetic denervation render improbable the participation of an uncontrolled inhibitory neural agency. Furthermore, the inhibitory phenomena are abolished when the vagi are cut. These facts point to decrease of vagal activity as being the mechanism by which the reduced motility in the experiments under discussion was mediated.

Youmans and Meek (1937) state: "There is no indication that the vagal fibers play any part in gastro-intestinal inhibition thus produced" (by rectal stimulation). Most investigators have reached similar conclusions regarding inhibition of the bowel. The reasons for the divergence between my results and those of other workers is not clear, but a number of possible causes for the discrepant findings may be advanced. 1. Differences in anesthetics may account in part for the conflicting data. 2. Some workers employed drugs, e.g., atropine (Bayliss and Starling, 1899; Bunch, 1899) and curare (v. Lehmann, 1913) which may have prevented elicitation of the reflex. 3. Different types of afferent stimulation have been used by various experimenters, and hence the conditions were not the same. 4. Perhaps most important in considering the discordant findings is the experimental animal employed. The majority of studies have been carried out on dogs, and the species differences between the cat and dog may well afford a possible solution of the discrepancies.

The results of central vagal stimulation are not so conclusive as those occurring after sciatic nerve excitation. In contrast to v. Lehmann (1913),

I found that augmentor effects always followed when the other vagus was intact (fig. 4). It is possible that v. Lehmann's inhibitory effects might have been due to his use of stronger induction shocks than those employed in these experiments. He states that more intense stimuli are required to bring on inhibition than augmentation. Since the adrenals were not removed in his experiments, the possibility of humoral effects was not excluded.

The variable response elicited by central vagal stimulation when the splanchnics alone are present is puzzling. M'Crea, M'Swiney and Stopford (1925) state that the results of direct stimulation of the vagus depend on the condition of the gastro-intestinal musculature at the time of the stimulation. When the smooth muscle is in a state of high activity vagal excitation may cause inhibition, while augmentation results if the peripheral structures are in a condition of lowered activity. The possibility of a similar situation existing when intestinal reflexes are set up is not excluded, but the present experiments offer no indication as to the validity of this supposition.

The appearance, in 4 cases, of enhanced motility mediated by splanchnic depression (fig. 5) indicates the possibility of obtaining a reciprocal synergism between the motor and inhibitory systems, since effects mediated by agents other than the splanchnic nerves were ruled out (p. 647).

One further point may be mentioned regarding the relative ease of obtaining evidence of reciprocity for inhibition and the difficulty of securing similar proof for augmentation. In the normal animal conditions of stress may arise not infrequently and require an efficient and rapid mechanism for initiating a decrease in the activity of the alimentary tract; then both efferent systems are correlated to occasion the most economical production of the standstill of the gastro-intestinal canal. It should also be emphasized that humoral agencies (adrenaline and sympathin from distant sources), which begin their action after a longer latency and have more prolonged effects, undoubtedly coöperate with the neural mechanisms in keeping the activity of the bowel depressed when conditions which demand its quiescence arise. On the other hand, situations which require increased motility of the intestines may be accomplished in a more leisurely fashion and need not requisition the aid of both nervous pathways. A reciprocal action in the latter case is not precluded; indeed, the evidence is suggestive that it actually may take place. The implication is simply that in the latter situation reciprocity is not so highly developed as when the gut must be inhibited to meet the exigencies of normal existence.

<div align="center">SUMMARY</div>

1. The movements of the small intestine were studied by the balloon method in cats and rabbits under urethane or dial.

2. The vagi exert a tonic motor influence on the small intestine (fig. 1; p. 644).

3. Faradization of the central stump of the sciatic nerve usually causes intestinal inhibition and a loss of tone. The decline in activity takes place even after the splanchnic nerves are destroyed (fig. 2). The diminished motility is shown to be due to a decrease in the normal activity of the vagi—hence, reciprocal innervation (p. 645).

4. In rare instances increase in movements and tone follows sciatic stimulation (fig. 3).

5. Central excitation of one vagus, with the other vagus and splanchnics intact, produces enhanced motility (fig. 4).

6. When both vagi are removed and the splanchnics are intact, central stimulation of one vagus causes either augmentor (fig. 5) or inhibitory reflexes. These are shown to be mediated by the splanchnic nerves (p. 647) and the augmentor reflex is advanced as possible evidence of reciprocal innervation (p. 649).

7. The significance of reciprocal innervation in the gastro-intestinal canal is briefly discussed (p. 649).

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DARK ADAPTATION AND EXPERIMENTAL HUMAN VITAMIN A DEFICIENCY[1]

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I. REASONS FOR THIS RESEARCH. The use of a visual function like dark adaptation to record the vitamin A state of the organism rests on two bodies of knowledge. The first is the evidence accumulated in the last 15 years which shows that vitamin A is the critical agent in the long-established association of night blindness with dietary irregularities. Night blindness in rats may be produced by a diet deficient in vitamin A, and this condition is cured by ingestion of vitamin A (Holm, 1925). Moreover, there is less visual purple formed in the eyes of A-deficient animals (Tansley, 1931), and it is regenerated more slowly during dark adaptation (Fridericia and Holm, 1925) than in normal animals. In human beings night blindness has been experimentally produced by an A-deficient diet (Jeghers, 1937; Hecht and Mandelbaum, 1938; Wald, Jeghers and Arminio, 1938) and the condition has also been reversed by resumption of a normal diet.

The second line of work is more recent, and has demonstrated that vitamin A is an essential ingredient of the chemical makeup of the visual system. Vitamin A is found in the retina (Wald, 1933), and appears as an ultimate product of the bleaching of visual purple (Wald, 1935). Thus vitamin A is a precursor for the formation of the visual pigments and is also a product of their decomposition,—a cycle which accounts for its necessity in vision, and which renders reasonable the employment of visual tests for the estimation of vitamin A in the body.

The visual test most commonly used for this purpose is some aspect of dark adaptation. However, in spite of its widespread clinical use in the diagnosis of human vitamin A deficiency, the precise behavior of dark adaptation during experimental vitamin A deficiency has not been established. This is first because only a few people have actually been studied experimentally, and second because even among these, differences have appeared in the experiments of different laboratories. The differences concern *a*, the time when a rise of the visual threshold first appears during the period of an A-deficient diet; *b*, the effect of single in-

gestions of vitamin A or of carotene on the visual threshold following a deficient diet, and c, the rate of recovery of the visual threshold after the resumption of a normal diet.

In order to supply material for the elucidation of these differences, we have made experiments in human vitamin A deficiency with 17 male subjects between 21 and 30 years old. Of these, 4 were studied during 1937–38 and have already been reported on briefly (Hecht and Mandelbaum, 1939); the other 13 were studied during 1938–39. The subjects were maintained on controlled diets for different periods and their dark adaptation was followed at frequent intervals.

II. COURSE OF DARK ADAPTATION. The precise characteristics of dark adaptation depend on many factors such as the retinal location, the color and the size of the test area, as well as the duration and extent of the preceding light adaptation (Hecht, 1937). In order to maintain these factors constant, we used the apparatus devised by Hecht and Shlaer (1938) and the standard procedure recommended by them. This has the advantage not only of furnishing data whose characteristics possess physiological meaning, but of enabling the data to be related to the normal distribution of adaptation properties which we have already studied with a population of 110 individuals (Hecht and Mandelbaum, 1939). The procedure involves light adaptation to 1500 millilamberts for 3 minutes, and measurements during the subsequent darkness of the threshold of a retinal area 3° in diameter situated 7° nasally for flashes of violet light 0.2 second in duration.

For purposes of illustration, the course of dark adaptation of subject F. W. is given in figure 1. The lowest points and curve record the subject's performance during his period on a normal, balanced diet, and are typical of all the other subjects. As always, the course of adaptation shows two stages which are now well understood in terms of the double nature of human vision. The primary rapid drop in threshold is generally recognized as cone adaptation, while the secondary, slower and more extensive drop in threshold is the expression of rod dark adaptation.

The other measurements in figure 1 were made at different times during the subject's stay on a vitamin A-deficient diet. They show that the form of the adaptation curve remains essentially the same, but that the two parts of the curve become displaced upward in threshold. The cone threshold does not rise as much as the rod threshold, though their course is approximately parallel. This has already been found by us before (Hecht and Mandelbaum, 1938), and is confirmed by the work of Wald, Jeghers and Arminio (1938), of McDonald and Adler (1939), and of Wald and Steven (1939).

Because of this upward displacement of the adaptation curve as a whole, the best single criterion of visual sensibility under these conditions is the

final rod threshold, usually reached in about 30 minutes of darkness. In presenting the results of our dietary experiments we shall give only this final rod threshold.

It should be pointed out that though this is an adequate treatment for the present experiments, there are circumstances when such a single datum is an inadequate expression of the changes in dark adaptation. An example is the progress of dark adaptation during alcoholic liver cirrhosis (Haig, Hecht and Patek, 1938; Patek and Haig, 1939) when not only the final cone and rod thresholds change, but the two segments of the
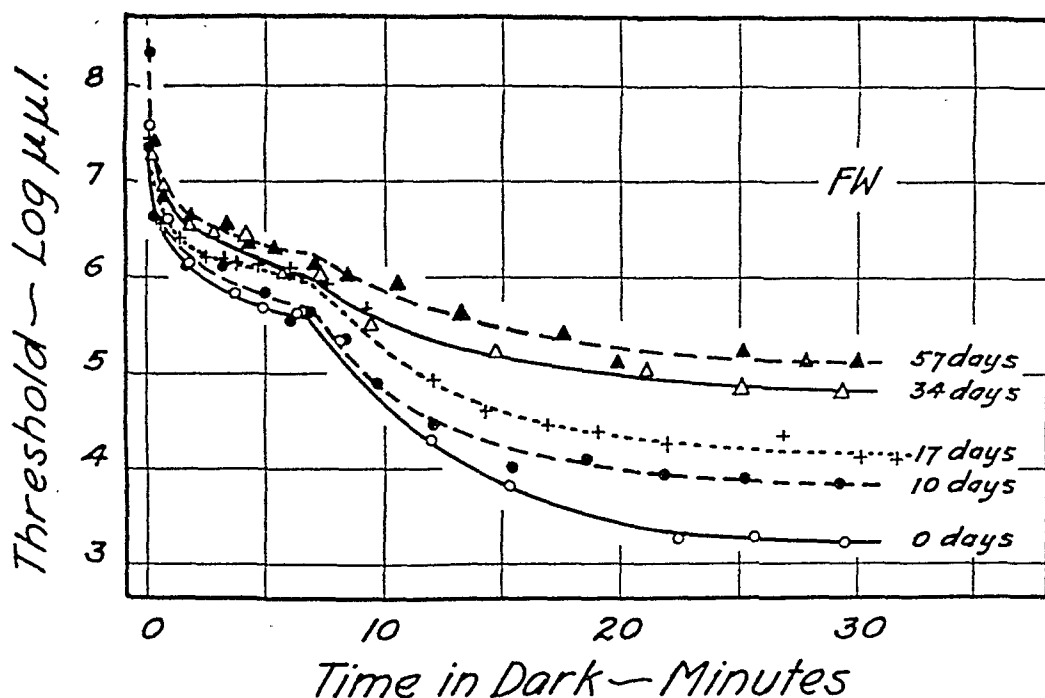
Fig. 1. The course of dark adaptation of a typical subject on a normal diet and at different times on an A-deficient diet. The ordinates are the threshold intensities given in log micromicrolamberts. The rod threshold at 30 minutes shows the largest effect of the diet and because it may be established at leisure, furnishes the best single criterion of the change during the diet.

curves shift relative to each other so that cone-rod transition time undergoes a steady change as well.

The curves in figure 1 show the futility of using a single point in the early stages of dark adaptation as a criterion of visual behavior. Clinical instruments like the biophotometer (Jeans and Zentmire, 1934; Jeghers, 1937) and the one described by Pett (1939) rely on rapidly made readings in the first stages of dark adaptation. Clearly, because of the great speed of adaptation at the beginning, measurements in this region are most difficult to make with precision, and are therefore least reliable. More-

over, the changes which the threshold undergoes during the A-deficient diet are least near the beginning of adaptation. It is hardly surprising therefore that such measurements are subject to statistical anomalies (Isaacs, Jung and Ivy, 1938) which frequently make them useless (Palmer and Blumberg, 1937). Conclusions drawn from results with instruments and procedures of this kind should be viewed with critical skepticism. It is to be hoped that whenever possible in clinical or dietary studies complete dark adaptation curves be made so that the precise visual effects may become known.

III. VITAMIN A DEFICIENCY AND VISUAL THRESHOLD. The first difference which has arisen in the work of different investigators concerns the time of appearance of changes in visual sensitivity following the adoption of a vitamin A-deficient diet. With 4 subjects placed on a deficient diet we found an immediate and rapid rise of cone and rod threshold (Hecht and Mandelbaum, 1938). With their one subject, Wald, Jeghers and Arminio (1938) similarly reported an immediate rise in threshold, which has since been confirmed on an additional subject by Wald and Steven (1939). However, other investigators secured no such immediate rise, and some found none at all. Thus the 5 subjects used by Booher, Callison and Hewston (1939) required 16, 27, 29, 39 and 124 days respectively on a vitamin A-deficient diet before showing a recognizable rise in threshold. Moreover, Steffens, Bair and Sheard (1939) report only slight and temporary rises in threshold for 3 subjects on a deficient diet for about six months. In fact, Wald and Steven state briefly that some of their subjects failed to show any rise in threshold when placed on the same regime as the subject which responded sharply.[2]

From our own experiments we can say that the work of all these investigators is undoubtedly correct. There is an astonishing variability in the behavior of individuals, and our records can duplicate all the results previously reported.

a. Experimental procedure. The procedure used was essentially the same with all of our subjects. While they were on their normal diets we measured their dark adaptation at frequent intervals (daily or every other day) for about two weeks. After their normal threshold level was established, the subjects were put on a diet which was restricted to foods calculated from the tables of Daniel and Munsell (1937) to yield about 150 International Units of vitamin A daily. Their dark adaptation was measured frequently during the diet and the behavior of the threshold recorded in relation to the diet.

[2] Doctor Wald in a personal communication has recently amplified this. Of 4 additional subjects who have been kept on the deficient diet, 3 failed to show any rise in rod threshold after 2 months, and one subject has shown no threshold rise even after 4 months.

The 17 subjects on the deficient diet were treated in three groups as shown in table 1. The first group of 4 subjects received no supplements of other vitamins during the A-deficient dietary period. The second group of 11 subjects received as daily supplements 125 units each of vitamins B_1 and G in the form of brewer's yeast, and 1800 of vitamin D as irradiated ergosterol. In addition each subject had at least 300 cc. skimmed milk daily, and was encouraged to eat grapefruit for vitamin C. The third group of 2 subjects received daily supplements of 200 units of

TABLE 1

Effect of a vitamin A-deficient diet on the visual thresholds of 17 young men

GROUP	DAILY SUPPLEMENTS	SUBJECT	INITIAL THRESHOLD LOG $\mu\mu l$	DAYS ON DIET	TOTAL RISE IN THRESHOLD LOG $\mu\mu l$
I	None	H. M.	3.00	43	0.90
		J. M.	2.80	43	1.10
		J. Mi.	3.15	36	0.90
		L. W.	2.85	36	2.05
II	125 units B_1	J. B.	2.95	60	1.05
	125 units G	F. C.	3.10	47	0.55
	1800 units D	D. J.	3.10	124	1.60
	300 cc. skimmed milk	S. K.	2.90	103	1.05
		H. L.	2.65	59	1.00
		W. L.	2.75	94	2.00
		D. R.	2.65	43	1.15
		F. S.	2.95	42	0.70
		J. S.	3.30	109	1.70
		W. S.	3.15	103	1.00
		F. W.	3.15	82	2.15
III	200 units B_1	B. R.	3.15	37	1.00
	200 units G	H. S.	3.05	38	1.15
	2000 units D				
	50 mgm. C				
	300 cc. skimmed milk				

B_1 and G, 2000 units of D, and 50 mgm. of ascorbic acid, as well as at least 300 cc. of skimmed milk.

b. Results. Figure 2 shows the rod thresholds for each subject during the normal diet period and during the deficiency diet period. The subjects have been arranged in a rough order corresponding to the speed with which the threshold rose during the deficiency period. The beginning of this period is marked with a heavy vertical line.

Out of the 17 subjects, 14 responded at once to the diet with an unmistakable rise in threshold. The other 3 subjects need separate comment.

F. C.'s threshold rose so slowly over a period of 45 days that one can hardly be sure of it; his experimental diet was terminated for extraneous
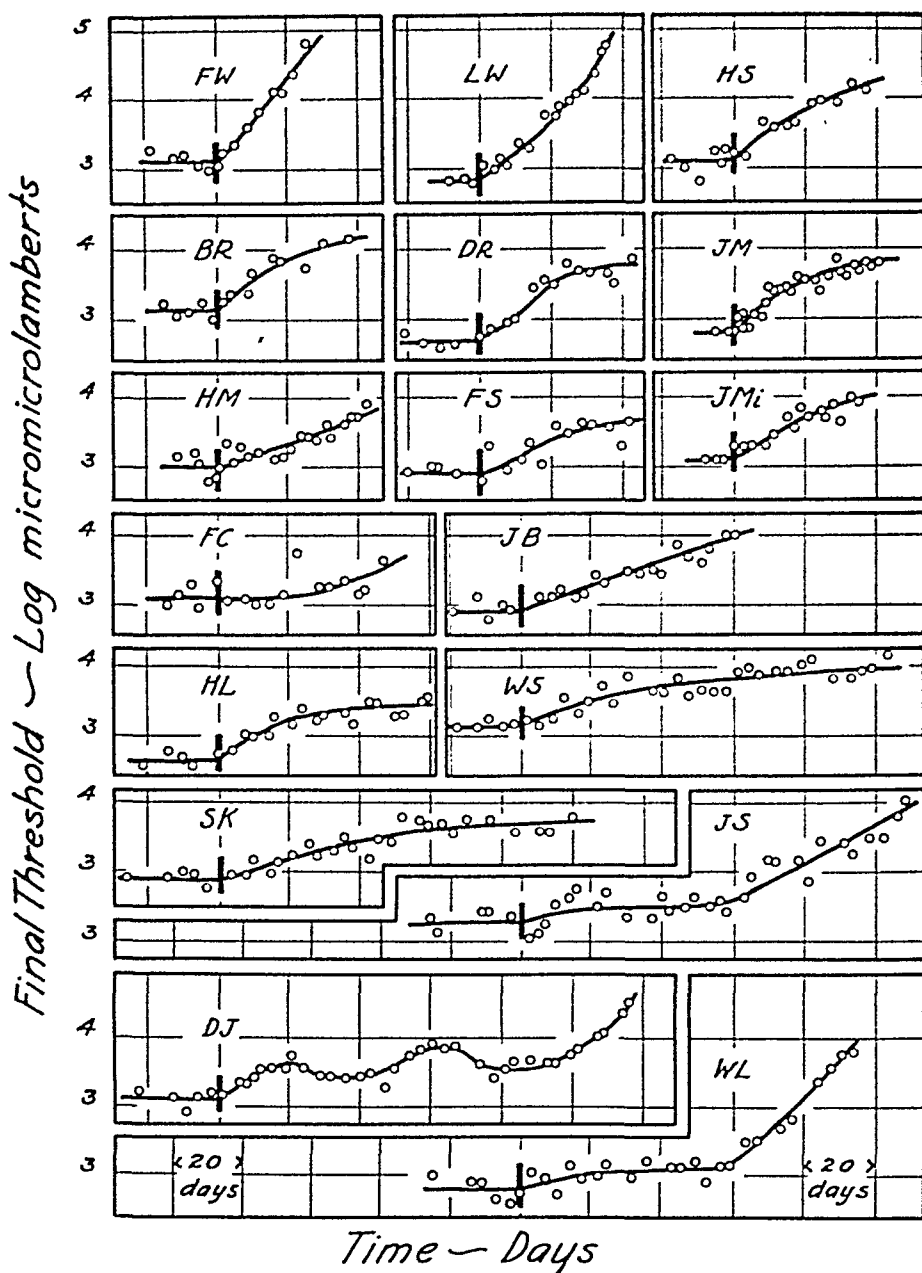
Fig. 2. Effect of a vitamin A-deficient diet on the 30 minute rod threshold of 17 young men.   The black vertical line marks the beginning of the diet.   In most cases the threshold begins to rise at once.

reasons.   J. S.'s threshold hardly rose over a period of 60 days, and then suddenly began to rise sharply and unmistakably.   An almost identical course was followed by W. L., who showed a very doubtful rise for about

60 days, and then suddenly began to rise in threshold with a speed comparable to the most spectacular behavior such as that of F. W. or L. W. One would hardly have blamed an experimenter for terminating the procedure with J. S. and W. L. after six or seven weeks and concluding that the visual threshold shows no change on a vitamin A-deficient diet.

Subject D. J. resembles in some ways those reported by Steffens, Bair and Sheard. On the deficient diet his threshold first rose unmistakably; but it soon stopped and even dropped, only to go through another cycle of up and down. By the end of 3 months, though the threshold was about 0.5 log unit above the initial normal, one would have hesitated to ascribe the rise to the diet, and one could have terminated the experiment as doubtful or even negative. However, at the end of the third month the threshold began to rise rapidly and extensively in the typical way already reported.

Supplementation of the A-deficient diet with other vitamins does not appear to influence the behavior of the threshold. Of the two subjects showing the most immediate responses, F. W. was in group II and received vitamin supplements, while L. W. received none. Similarly out of the next group of 7 subjects showing about the same degree of response, 3 were in group I, 2 in group II, and 2 in group III. Moreover, the 3 subjects who showed no immediate effects of the diet were all in group II which received adequate supplements of the other vitamins.

The rise in threshold is unrelated to the initial normal threshold. This is evident by simple inspection. However, we computed the coefficient of correlation between the initial threshold and the extent of the rise after 36 days on the deficient diet. The coefficient is -0.25 which is negligible. Similarly the correlation between the initial normal threshold and the average rate of threshold rise also yields an insignificant coefficient, -0.31.

The rate of the rise in threshold during an A-deficient diet may be taken as a measure of the vitamin A status of an individual. If this is so, then the lack of significant correlation between initial threshold and rate of rise during the deficient period indicates that within the normal range the threshold level itself can not be taken as an index of the nutritional state of an individual. This confirms our previous study (Hecht and Mandelbaum, 1939) of a university population of 110 individuals.

c. *Subjective symptoms.* In contrast to the reports by Jeghers (1937) and by Pett (1939), only two of our subjects recorded subjective awareness of their raised visual thresholds. The subjects are F. W. and L. W., and as figure 1 shows, they gave the most extreme and most immediate visual response to the deficient diet. At the very end of his deficient period L. W. reported an accidental collision with a wall at home due quite obviously to his raised visual threshold. Similarly F. W. near the end of

his deficient period observed that a longer interval of waiting in the fluoroscopy room was necessary before he could make observations.

None of our subjects reported any skin symptoms, and we observed none. There were no signs of epithelial keratinization of the eye, which confirms a similar lack of findings by Booher, Callison and Hewston, even on their subject whose threshold rose 3.5 log units on an A-deficient diet. In the light of our experience the report by Pett (1939) of anorexia, mild diarrhea, sore throat, rhinitis, decrease of salivary flow, gingival ulcers, dry, scaly skin, and painful eyes, all in one person on an experimental A-free diet must be considered as exceptional.

d. Conclusion. From our results it is fair to conclude that in most cases (14 out of 17) when a person is put on a vitamin A-deficient diet his visual threshold will rise almost immediately and will continue to rise so long as the diet is maintained.

The failure of some individuals to respond for as long as three months may be due to an exceptional storage of vitamin A in the tissues. If this is so, then the reserve can be drawn upon for long periods without signs of visual deficiency. When, however, a threshold change does begin to appear, the rate of change, and therefore the rate of storage depletion, is quite rapid.

Storage capacity for vitamin A apparently has no relation to the amounts recently ingested. Steininger, Roberts and Brenner (1939) found it to depend more on individual capacity and on long term history. This is borne out by the experience of Wald and Steven, who gave all their subjects a short period of high vitamin A dosage, and still got widely different individual effects with the subsequent deficient diet.

IV. SINGLE DOSES OF VITAMIN A. The second difference which has appeared in work from different laboratories concerns the effectiveness of single large doses of vitamin A in restoring the visual threshold after its rise on an A-deficient diet. There have been clinical reports of night blindness which has been cured within 24 hours by single oral administrations of a large quantity of vitamin A (*e.g.*, Aykroyd, 1930; Lewis and Haig, 1940), as well as by intramuscular injections of vitamin A concentrates (Edmund and Clemmesen, 1936) in even shorter time. In addition, Jeghers recorded the rapid recovery from experimental night blindness following the eating of large quantities of vitamin A. The most dramatic data are those of Wald, Jeghers and Arminio (1938), and of Wald and Steven (1939) obtained with a single subject each, in which the return of the visual threshold to normal occurs within minutes after ingestion of even moderate concentrations of vitamin A and of carotene.

Other investigators have not been able to secure similarly rapid recoveries. We (Hecht and Mandelbaum, 1939) obtained only slight effects with two subjects whose threshold had risen 1 and 2 log units above

normal as a result of a deficient diet, when they received oral doses of vitamin A concentrates containing 100,000 units. Similarly McDonald and Adler (1939) were unable to influence with single doses the visual threshold of their one subject which had risen on a deficient diet. Essentially the same findings are reported by Steffens, Bair and Sheard.

Our two subjects referred to above differed from those of Wald and his co-workers in having received no supplements of the other vitamins during their A-deficient period, and we were inclined to attribute the differences to this. We have now studied 7 additional subjects, all of whom had received supplements of other vitamins; 2 were in group III, and 5 in group II. The results do not bear out such an hypothesis.

a. Procedure. The experiments were made in two ways. A subject whose threshold was high as the result of an A-deficient diet had his dark adaptation measured in the usual way. When the threshold levelled off after 30 or 40 minutes, the subject, while still in the dark, was given a single dose of *oleum percomorphum* or of a concentrate containing a high value of vitamin A. During the next few hours the subject remained in the dark, and at frequent intervals his threshold was determined in order to see whether the ingested vitamin A produced any effects.

The first experiments with this method produced a good deal of boredom but little change in threshold in the course of several hours. The method was therefore abandoned for the following one. The subject had his dark adaptation measured in the usual way. He was then given a large, dose of vitamin A and permitted to do what he wanted in the laboratory. Every hour or so after this, his dark adaptation was again measured with the standard procedure and the adaptation curves compared with the one secured before the single dose had been taken.

b. Results. The outcome was disappointing. Not one of our subjects, two of whom we tested on three separate occasions, responded with a complete return of the threshold to normal. However, most of our subjects, as the result of single large doses of vitamin A, showed some drop in threshold which varied in extent among the different individuals.

Figure 3 illustrates the course of some of the experiments. The solid circles connected by a vertical line represent the maximum change in threshold following single ingestions of about 100,000 units of vitamin A. The greatest effects were secured with D. J. and F. W., but in neither instance did the threshold return even half way to normal (on a log intensity scale).

The three successive trials with F. W. are significant in showing that on the day following each ingestion the threshold came back to its previous high position. Thus the effect of these single doses was temporary and cannot be considered as "cures" of night blindness.

Table 2 gives a summary of the findings with the 9 subjects. It is
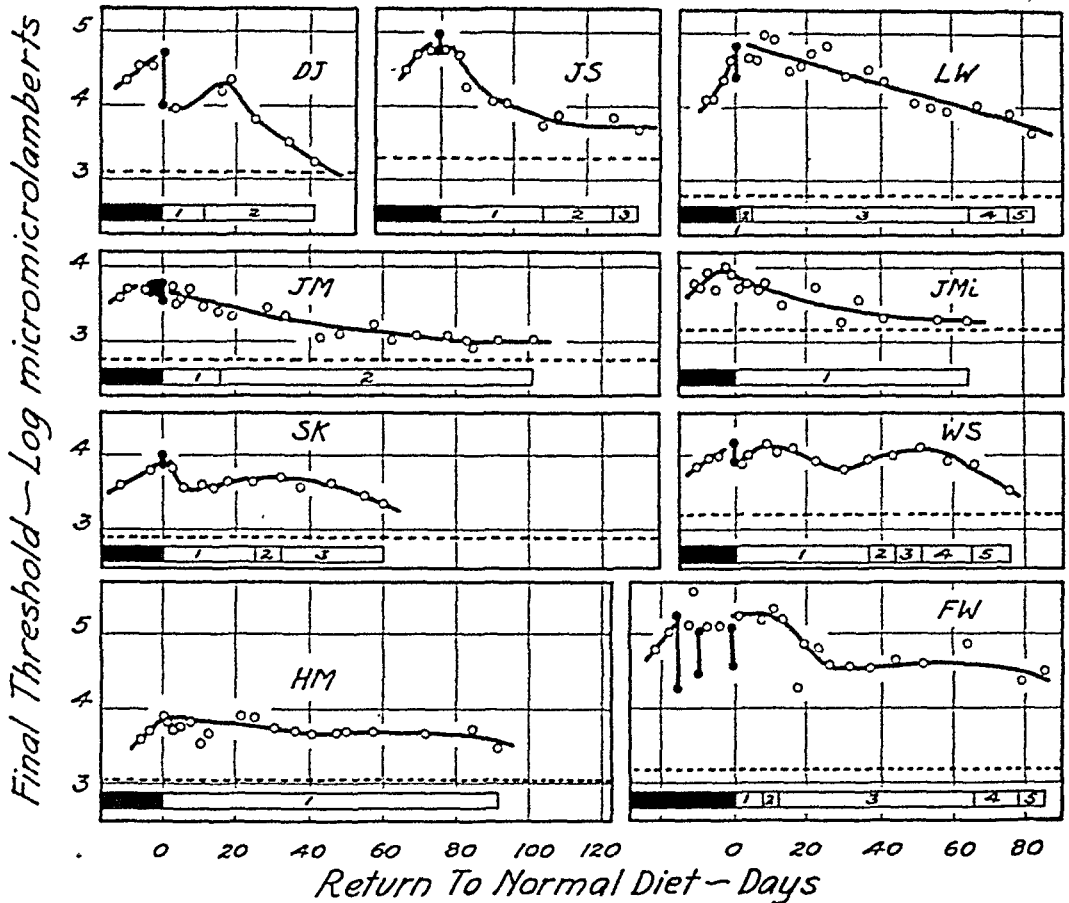
Fig. 3. Recovery from a vitamin A-deficient diet. In each case the dotted line represents the original threshold before the deficient diet. The black band marks the final part of the deficient diet, and the last few of the measurements of the deficient period are shown. The dark circles connected with heavy vertical lines record the effects of single ingestions of large doses of vitamin A. The clear band represents the return to a normal adequate diet. The numbers in this band indicate the following daily supplements:

D. J. (1) 25,000 units vitamin A; (2) 60,000 units A, 200 units B_1, 200 units G, 2 oranges.

J. S. (1) 25,000 units A; (2) no supplements; (3) 60,000 units A, 200 units B_1, 200 units G, 50 mgm. C.

L. W. (1) 50,000 units A; (2) no supplement—ill; (3) and (4) same as 1; (5) same as 1 plus 30 grams bile salts.

J. M. (1) 50,000 units A; (2) 50,000 units carotene.

J. Mi. (1) no supplements.

S. K. (1) 25,000 units A; (2) 60,000 units A; (3) same as 2 plus 200 units B_1, 200 units G, 2 oranges.

W. S. (1) 25,000 units A; (2) same as 1 plus 120 units B_1 and G; (3) same as 1 plus 200 units B_1 and G; (4) 50,000 units A, 200 units B_1 and G; (5) 120,000 units A, 200 units B_1 and G.

H. M. (1) no supplements.

F. W. (1) 25,000 units A; (2) no supplements; (3) same as 1; (4) 40,000 units A, 200 units B_1 and G; (5) 100,000 units A, 200 units B_1 and G.

apparent that the responses to single doses are as variable as were those to the deficient diet. The largest decrease in threshold is 1.00 log unit; the smallest is 0.05 log unit, which is really no effect, since a change of threshold of less than 0.15 log unit cannot be considered as reliable. The extent of the temporary threshold recovery does not seem correlated with the time during which the subject had been on the deficient diet, nor with the size of the threshold rise produced by the deficient diet, nor indeed with the supplementary vitamins given during the diet.

In one case (D. J.) we gave not only a high concentration of vitamin A but bile salts to aid in emulsification and absorption. The subsequent

TABLE 2

Effect of single large doses of vitamin A on the visual threshold of subjects on a vitamin A-deficient diet

GROUP	SUBJECT	DAYS ON DEFICIENT DIET	TOTAL RISE OF THRESHOLD ABOVE NORMAL LOG $\mu\mu$l	UNITS OF VITAMIN A IN SINGLE DOSE	MAXIMUM DROP IN THRESHOLD LOG $\mu\mu$l	TIME FOR APPEARANCE OF MAXIMUM DROP
						hours
I	J. M.	41	1.10	11,000	0.05	8
		42	1.10	30,000	0.10	8
		43	1.10	50,000	0.20	8
	L. W.	35	2.00	100,000	0.40	6
II	D. J.	124	1.60	100,000*	0.70	$3\frac{3}{4}$
	S. K.	103	1.05	100,000	0.11	5
	J. S.	109	1.70	100,000	0.23	$3\frac{1}{2}$
	W. S.	103	1.00	100,000	0.28	5
	F. W.	68	2.15	100,000	1.00	$3\frac{1}{2}$
		73	1.90	100,000	0.55	3
		82	2.00	100,000	0.55	$3\frac{1}{2}$
III	B. R.	37	1.00	150,000	0.27	6
	H. S.	38	1.15	400,000	0.38	6

* Plus bile salts.

reduction in threshold was no greater than without bile salts in other instances, as for example with F. W.

In short, the reasons for the differences between our results and those secured by Wald still remain to be discovered. In the light of all the variation encountered, it is likely that Wald's two subjects may have been, by a curious statistical twist, extreme variates in an ordinary population distribution.

V. RECOVERY. The occasionally dramatic effectiveness of single doses has given the impression that visual recovery from an A-deficient diet is a matter of hours or perhaps days. Our previous experience showed that

it required at least 2 months after resumption of a normal diet with or
without supplementation of vitamin A for a return to normal. Similar
reports have been made by McDonald and Adler, and by Steffens, Bair
and Sheard. Our present experiments further confirm these findings.

We were able to follow for over two months the history of 9 out of the
17 subjects who had been on a deficient diet, and who had returned to a
normal diet containing about 10,000 units of vitamin A. During this
subsequent normal period the subjects received a variety of treatments
ranging from no supplements at all to generous additions of vitamins A,
B_1, G, and C. The details of these supplements are given in the legend
to figure 3.

Figure 3 shows that there is great variation among the subjects during
recovery. Only 3 individuals (D. J., J. M., J. Mi.) recovered completely
during the period of observation. We consider a recovery as complete
when the threshold returns to within 0.3 log unit of the initial normal
threshold; this is because a range of 0.3 log unit is not uncommon in the
threshold of a single person measured over a long period of time. Of the
remaining subjects, 4 (J. S., L. W., S. K., W. S.) were obviously on the
road to recovery, while 2 (H. M., F. W.) showed a persistently elevated
threshold throughout the three months of observation.

The most significant point which these data show is that *visual recovery
from an A-deficient diet is a matter of weeks and months, and not of hours
and days.* The fastest recovery was made in 6 weeks; the slowest was
not complete after 3 months despite the fact that in one instance (F. W.)
the supplementary daily dosage of vitamin A was raised to 100,000 units
and that other vitamins were given in addition.

It is necessary to add that even these extremely slow individuals ulti-
mately recover. A year after the experiment was terminated we were
able to measure J. M. who had practically reached normal after 3 months
and F. W. whose threshold had remained 1.2 log units above normal even
after the same time. In the subsequent year both had become completely
normal. In fact F. W's. threshold, which had been so alarmingly high,
had dropped even below its original normal level.

The 4 subjects who recovered most rapidly illustrate the difficulties of
drawing conclusions about the phenomenon. Of the two who recovered
fastest (J. Mi., and D. J., 40 and 41 days respectively) one (J. Mi.) had
received no supplements of any kind either during the A-deficient period
or during the recovery period, while the other (D. J.) had received supple-
ments of the other vitamins during the deficient period, and of A and the
other vitamins during the recovery period. Of the other 2 subjects J. M.
had received no supplements during the deficient period and 50,000 A units
daily during recovery, while J. S. had received supplements of the other
vitamins during the deficient period, and none during recovery (except

for the last few days). In other words, on the basis of these experiments one cannot find any effects of special dietary supplements on recovery rate. The recovery rate from a vitamin A-deficient diet almost seems an inherent property of the individual.

It is of course possible that we have missed some critical factor in all this procedure. We are inclined to doubt this because of the large variation shown by individuals during the period of the deficient diet. If individuals vary so much in their rate of depletion, it is not unreasonable to assume that they also vary in their rate of recovery. In any case, the important thing to emphasize is that recovery from vitamin A deficiency is a very slow process and that dramatic recoveries in short times are exceptions rather than the rule.

SUMMARY

1. Measurements of the dark adaptation of 17 young men made before, during, and after their stay on a vitamin A-deficient diet show that cone and rod visual functions are both affected by the diet. The cone thresholds rise less than the rod thresholds, but their behavior is approximately parallel. The best single criterion of these changes is the final rod threshold after 30 minutes in the dark.

2. When subjected to the deficient diet, most individuals (14 out of 17) responded by an immediate and unmistakable rise in visual threshold which continued for the duration of the diet. The other 3 subjects required 22, 55 and 60 days respectively before giving a clear and continuous rise in threshold. None of the subjects showed any skin symptoms, and only the two most extremely affected individuals recorded a subjective awareness of their raised visual thresholds.

3. Partial recovery of the visual threshold was accomplished in some individuals by single large doses of vitamin A. The extent of the recoveries was variable. The recoveries were never complete and were always temporary.

4. Permanent recovery from an A-deficient diet was slow, requiring weeks and months. The fastest subject returned to normal in 6 weeks; the slowest maintained a high threshold for over 3 months, but had recovered completely after a year.

5. Efforts to influence the visual responses during and after the A-deficient diet by supplementation with other vitamins yielded only negative results.

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THE EFFECT OF TRACES OF TIN ON THE RATE OF GROWTH OF GOLDFISH

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The nature of the rôle of tin in animal and plant physiology is not well known. Although tin has been reported from time to time as occurring in traces in the mammalian body (3, 4, 14), it has not been commonly regarded as important. Fearon (6) in his classification of the elements according to their relations with life includes tin among the variable micro-constituents; but Godden (8) concludes that there is no evidence to indicate that it exercises any useful function in animal nutrition. Richardson (13), on the other hand, regards tin, along with rubidium and aluminum, as accessory factors in animal nutrition, and adds that the physiological significance of these is still imperfectly understood.

The present report is an outgrowth of long and carefully executed laboratory studies on the rate of growth of goldfish in which it has been found that these animals grow somewhat, yet significantly, faster in water in which other goldfish have previously lived, for a limited period, than in otherwise uncontaminated water (1, 2). The last paper on this work (2) indicated that when the synthetic pond water, in which the fish lived, was made up with water from an aluminum-lined still, the growth results differed appreciably from those obtained when the water from a block-tin-lined still was used. Briefly, in the latter case, there was a greater growth of both the control fishes in uncontaminated water and of the experimental fishes in conditioned water, as against the growth of comparable groups in water from an aluminum-lined still; and also, the difference between the control and the experimental groups was significantly greater when the tin-lined still was in use.

In order to test the conclusion that tin, probably in ionic form, may have been responsible for these results, the present set of experiments was carried out. Here, stannous chloride was added in trace concentrations to the synthetic medium used for the fishes. This medium is a "synthetic pond water" developed in this laboratory for work with fishes and other aquatic forms. It consists of distilled water, in this case from an aluminum-lined still, to which salts of analytical quality are added in

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following quantities per liter: 100 mgm. $CaCl_2 \cdot 4H_2O$, 50 mgm. $MgSO_4 \cdot 7H_2O$, 50 mgm. K_2SO_4, and 50 mgm. $NaNO_3$. It was found, empirically, that a concentration of $SnCl_2$ of the order of magnitude of 0.000005 M would not increase the specific conductance of stock distilled water (with a value of 2×10^{-6} reciprocal ohms) beyond our arbitrarily set upper limit for "good" distilled water of 4×10^{-6} reciprocal ohms. Concentrations of tin up to this strength might conceivably have been present in the water during experiments when the tin-lined still was in use.

The methods for handling the fish have been described in detail elsewhere (1, 2). In summary, immature single-tailed goldfish (*Carassius*

TABLE 1

Increase in growth of fish; analyzed by experiment

EXPERIMENT (1)	CONCENTRA-TION 10^{-6} M (2)	INCREASE IN LENGTH			NUMBER OF FISH	
		Tin (3)	Control (4)	Difference (5)	Tin (6)	Control (7)
F20a*	4.2	0.73	0.41	0.32	9	10
F20b	4.2	1.17	0.45	0.72	9	9
F21a	4.2	0.89	0.85	0.04	9	10
F21b	4.2	1.22	1.04	0.18	10	10
F24a	4.2	2.56	1.69	0.87	15	14
F25a	5.0	0.75	0.61	0.14	19	18
F25a	2.5	0.43		−0.18	9	
F25b	5.0	0.72	0.60	0.12	17	19
F26a†	5.0	1.51	1.13	0.38	15	14
Mean...............		1.11	0.82	0.29	112	104

* "a" indicates that uncontaminated water was used as the medium, "b" that fish-conditioned water was used.

† Assay period of 14 days.

auratus) ranging in length from 30 to 45 mm. were studied for their growth during brief assay periods, usually 20 days. Photographs taken before and after each period were measured and the *increase in length* is reported, here in artificial units (1 unit = 0.82 mm.). The fish were kept singly in one-gallon, glazed earthenware jars, filled to 2 liters, and were changed daily to carefully washed similar jars containing freshly prepared water. The fish were usually fed during the last 3 hours of a 24 hour period so that, during each day, they were not in contact with food, except for the presence of minute regurgitated particles in the fish-conditioned water; otherwise they were in clean (food-free) water. The food consisted of ground dry Quick Quaker oats.

In some experiments, in connection with other work, fish conditioned water was used as a medium for the assay fish. The fish which were used to prepare this water were larger, averaging 70 mm. in length, and were kept in similar but larger jars which held 6 gallons of water, and these fish were also changed to fresh water daily and were fed in water other than that which they were conditioning.

The SnCl₂ was prepared in stock solutions of 0.01 M; this was added daily to the water to be conditioned and to the uncontaminated water, as the case may be, in appropriate amounts to secure the desired final concentration. No attempt was made to prevent the formation of stannic

TABLE 2

Increase in growth of fish; analyzed by paired fish

EXPERIMENT (1)	CONCENTRATION 10⁻⁶ M (2)	NUMBER OF PAIRS (3)	INCREASE IN LENGTH	
			Sum of differences by experiment (4)	Mean difference by experiment (5)
F20a	4.2	9	2.6	0.29
F20b	4.2	8	5.7	0.71
F21a	4.2	9	1.2	0.13
F21b	4.2	10	1.8	0.18
F24a	4.2	14	11.1	0.79
F25a	5.0	17	3.1	0.18
F25a	2.5	8	−1.1	0.14
F25b	5.0	16	2.1	0.13
F26a	5.0	14	6.0	0.43
Total.....................		105	32.5	
Mean.....................			0.31	

ions, or to control the appearance of insoluble hydroxides by the use of acid since it was not desired to change the customary pH relations. The white suspension which began to appear in the stock solutions at about the 10th day of the experiment was simply stirred and added to the synthetic pond water as the stannous chloride had been previously.

The data were analyzed for statistical significance by "Student" 's method.[1] This was done in two ways: *a*, the mean differences in increase in length between treated and control fish were tabulated by experiment and the mean of means was analyzed; *b*, the differences between pairs of fish, randomly coupled within each experiment, were averaged and this

[1] Statistical significance is measured by P, calculated by "Student" 's method, which is designed for situations where relatively small numbers of observations are involved. P = 0.05 is equivalent to 2 × the standard error, or 3 × the probable error, and is taken as the upper limit of statistical significance (7, 16).

mean of differences was tested.[2] The results of the analysis by both the paired experiment and the paired fish methods are shown in tables 1 and 2, respectively. From the former it will be seen that in 9 comparisons, by experiment, the fish in tin-treated waters grew, on the average, 0.29 units more, in the indicated assay periods, than did the control fish in tin-free water, and this is significant for $P = 0.0316$. When the case of 2.5×10^{-6} M concentration is omitted, the difference between the tin-treated and the control fish becomes 0.35, and since $P = 0.0132$ this is even more significant.

In table 2, where the same data are analyzed by the paired fish method, similar results are obtained. Here, the over-all difference is 0.31 and, since $P = 0.000318$, is to be taken very seriously. Again, when the atypical group with the 2.5×10^{-6} M concentration is omitted, the difference, now 0.35, has an even more significant P value of 0.000096. This means that there are only 96 chances in a million that this result would be obtained by random sampling.

It should be stated at this point that only in experiments F20b and F24a of both tables, and also in F20a in table 2, did the differences show significance when analyzed within each experiment. However, it will be seen, by reference to column 5 in each table, that in 8 of the 9 experiments there was a positive trend, and that this trend is significant is indicated in the preceding paragraphs.

From these data it may be concluded that tin in one or other of its ionic forms, probably the stannous, promotes growth in goldfish under experimental conditions. This conclusion supports the inference made in a previous paper (2) that the change from a tin-lined to an aluminum-lined still may have affected the composition of the distilled water sufficiently to account for the observed changes in growth which accompanied the transfer of stills. Specifically, it seems quite possible that the tin-lined still gave off enough tin, evidenced by the observed rapid corrosion, to affect favorably the rate of growth of the fish, and the absence of traces of tin when the aluminum-lined still was in operation resulted in a decreased rate of growth under a variety of conditions.

That traces of tin may contaminate distilled water is suggested by the work of Stout and Arnon (15) who report that ordinary distilled

[2] In a the formula $t = \dfrac{\bar{x}}{\sqrt{\dfrac{\Sigma x^2 - \bar{x}\Sigma x}{n(n-1)}}}$ was employed; here x represents the mean difference in each experiment (column 5), $\bar{x}$ the mean of means, and n the number of experiments. In b the same formula was used, but here x is the difference in increase in growth for each randomly chosen pair, $\bar{x}$ the mean of such differences, and n is the number of pairs. P is obtained from a table of probabilities for "Student" 's distribution.

water contained a variety of metal contaminants, varying from 0.1 to 0.01 part per million, and which were traced to tin-lined copper stills and to the metal piping through which the distilled water circulated. Earlier, in 1933, Hance (9), using spectroscopic analysis, found traces of tin, as well as of zinc, copper, lead, nickel, and chromium, in the distilled water prepared at the Hawaiian Sugar Planter's Experimental Station.

The stimulating properties of tin for biological systems are not unknown. Micheels and De Heen (12) and again Micheel (11) found that colloidal tin stimulated the germination and early growth of wheat, oats, peas, and buckwheat. Yoshida (17, 18) reported that colloidal tin, as well as the colloidal preparations of other metals, accelerated the growth of fibroblasts and of splenic tissue *in vitro* when present in moderate concentrations, while it inhibited growth at higher and had no effect in lower concentrations. Hotchkiss (10), working with suspensions of *B. coli communis*, found that for a large number of salts of heavy metals, each salt was toxic at some concentration, but that in many of these salts a concentration was found which stimulated the growth of the treated bacterial suspensions over that of the untreated control. $SnCl_4$ was found to be stimulating at 0.00005 to 0.000005 M. Similarly, Young (19) found that tin stimulated the growth of timothy when present at 100 parts per million, and of algae when the concentration was between 0.002 and 0.004 part per million. Cohen (5) also found that tin in concentrations of 0.01 and 0.05 parts per million, as stannic chloride, stimulated root growth in sunflowers.

It will thus be seen that the bulk of the work on the stimulating properties of trace quantities of tin has been done in the fields of botany. The present paper indicates a possible rôle of tin in the metabolism and growth of fishes. Tissue cultures aside, so far as we know, there is no record of any previous work with tin with similar results on any of the classes of vertebrates.

SUMMARY

1. Tin, as stannous ion, in concentrations of the order of 0.000005 M, has been found to accelerate the growth of goldfish, during brief assay periods, over that of untreated control fish.

2. This report extends, for the first time, the stimulating properties of traces of tin to the growth of vertebrate organisms.

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GROWTH AND DEVELOPMENT OF SIX GENERATIONS OF THYMECTOMIZED ALBINO RATS[1]

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Andersen (1932) in reviewing her own experiments and the studies of other workers upon thymectomized animals concluded that there is little or no relationship between the thymus and the gonads. In most of these studies thymectomies were performed in animals at least 20 days old. However, Andersen stated that in some of her rats the thymus was re-removed on the first day of life. Extensive data on these latter animals were not presented but the comment was made that no effects on vaginal canalization or development were noted.

Shay and his co-workers (1939) have since reported that irradiation of the thymus area in the 1 or 2 day old rat with massive doses of x-ray produced complete atrophy of the thymus. They also reported a re-tardation of growth in the males, an absence of sperm from the testes in animals 100 days old, and the presence of castration cells in the pituitary. However, if x-rayed males were permitted to live for 200 days they were essentially normal, i.e., spermatogenesis was resumed and castration cells disappeared from the pituitary.

Hashimoto and Freudenberger (1939) have criticized this work, but their data presented on thymectomized animals fails to clarify the issue since they used older animals (25 days). They feel, as we do (Nelson, 1939) that the thyroids must have been damaged by secondary irradiation in the experiments of Shay. It is unlikely that the thyroids were completely destroyed, since that would lead eventually to a complete cessation of growth (Salmon, 1938), but it seems probable that they were damaged enough to produce thyroidectomy cells which appear to be identical with castration cells (Nelson and Hickman, 1937; Severinghaus, 1937).

Dr. J. C. Plagge of the University of Chicago has kindly informed us that he has been unable to find any differences in the time of appearance of sperm heads between normal or sham operated controls and rats thymectomized at 1 day of age. Growth of both soma and gonads was

unaffected by the operation, a finding which held as well for the hormone output of the testis.

Putzu reported in 1934 that thymectomy in 5 successive generations of rabbits had no effect on growth or development. However, Einhorn and Rowntree (1936a, b; 1937) stated that thymectomy in successive generations of albino rats resulted in an accruing retardation of growth and development. This will be recognized as the reverse of results reported by these workers in successive generations of albino rats treated with
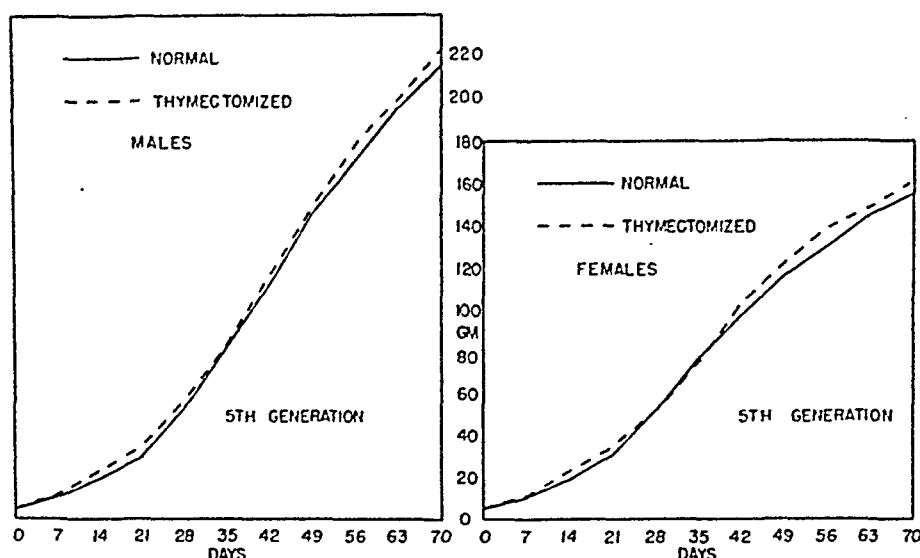
Fig. 1

TABLE 1

Development

SERIES	VAGINAL CANALIZATION			1ST ESTRUS	TESTES DESCEND	EARS OPEN	INCISORS ERUPT	EYES OPEN
	Weight	Body length	Days					
	grams	*mm.*		*days*	*days*	*days*	*days*	*days*
Normal.......	85 ±16.2	137 ±10.9	40 ±5.3	40 ±5.5	19 ±1.4	2.5	11.0	15.8
Thymectomy.	94 ±16.6	146 ±10.2	40 ±5.7	41 ±5.5	17 ±1.4	2.5	10.3	14.4

thymus extract (Hanson). Thymectomized animals which were treated with the extract grew normally or showed a slight acceleration (1938).

Chiodi (1938a, b) has reported that thymectomy in five successive generations of albino rats failed to alter the course of growth or development.

EXPERIMENTAL. All members of a litter of albino rats were thymectomized at 21 days and weaned at 25 days. This litter was used to initiate the experimental series. Thymectomies were performed uniformily at 21 days by the usual sternal approach. The method was essentially that of Einhorn (1936a), with the addition of tying in the end of the

thread for closure of the thorax before making the sternal incision. The operative mortality was approximately 5 per cent. Control animals were housed in adjacent cages under rigidly standardized conditions and were inbred to the same extent and over the same period as the experimental animals. All observations were made and recorded at the same time each day. The animals were fed our stock diet of Ti-O-Ga dog chow and water with the semi-weekly addition of lettuce. Animals which were not reserved to carry on the next generation were sacrificed at 70 days and the viscera were carefully weighed.

Although 6 generations have been studied, the extensive data for the 5th generation only are being presented since the larger number of animals

TABLE 2

Body weights and dimensions @ 70 days

MALES	NUM-BER	WEIGHT	BODY	TAIL	FORE LIMBS	HIND LIMBS
		grams	*mm.*	*mm.*	*mm.*	*mm.*
Normal.....................	25	216 ±21	195 ±6	165 ±9	160 ±4	210 ± 6
Thymectomy..............	17	225 ±13	197 ±5	171 ±7	152 ±11	203 ±13
Females						
Normal................:........	23	155 ±13	179 ±7	154 ±7	154 ± 6	195 ±13
Thymectomy..............	24	161 ±14	178 ±5	159 ±4	139 ±10	182 ±16

TABLE 3

Organ weights @ 70 days. Weights in milligrams

MALES	THYMUS	KIDNEY	ADRE-NALS	THY-ROID	PITUI-TARY	HEART	TESTIS	SEMINAL VESICLE	
								Full	Empty
Normal..........	477 ±68	1,050 ±156	35 ± 5	18 ±4	8.4 ±1.2	785 ±89	1,235 ±146	298 ±60	136 ±40
Thymectomy....		977 ±138	39 ±10	20 ±6	8.8 ±1.9	770 ±51	1,301 ± 93	295 ±67	144 ±30
Females........							Ovaries		
Normal	378 ±69	787 ± 98	45 ±10	17 ±3	9.8 ±1.8	612 ±58	59 ± 15		
Thymectomy ...		667 ± 79	44 ±17	16 ±3	8.3 ±1.2	563 ±64	58 ± 11		

in this group gives the data a greater statistical significance. However, these figures for the 5th generation might well apply to any of the other 5 generations.

An examination of the growth curves in figure 1 will show the complete absence of any retarding effect of thymectomy upon the rate of growth. Indeed, the thymectomized animals, both males and females, were slightly larger than their corresponding controls.

The absence of differences in the occurrences of developmental events is shown in table 1. The fact that the body length at vaginal canalization shows the smallest proportionate standard deviation of any of the criteria measured argues in favor of the reliability of the observations since Engle

and his associates have pointed out that of all the criteria which can be measured at vaginal canalization, body length show the least deviation.

The body weights and dimensions and the organ weights at 70 days (tables 2 and 3) for experimental and control animals show no differences as great as one standard deviation.

SUMMARY

Thymectomy on the 21st day of life in six successive generations of albino rats has not altered the rate of growth in males or females. Furthermore, in none of the developmental events which we have studied has there been any departure from the normal time of occurrence. In all, 69 males and 60 females were thymectomized, while 124 males and 139 females were studied as controls.

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CHANGES IN RESPIRATION ON INFLATION AND DEFLATION OF THE CHEST

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In one of the more recent studies of the effects of inflation and deflation of the chest (Hammouda and Wilson, 1932) alterations of pulmonary volume were obtained by varying the pressure around an animal in a body plethysmograph. The conclusions of these authors differ in several respects from those of previous workers. The observations to be reported in this paper were made by a similar method. They do not, however, support the more distinctive conclusions of Hammouda and Wilson as to the functions of afferent fibers in the vagus nerves. The difference in our conclusions is due in part to the introduction of another method of analyzing the reflex effects of the vagi, making use of measurements of resistance to inflation and deflation of the chest. In addition, some information is presented as to the effect of vagotomy on the carbon dioxide and oxygen content of arterial blood which has a bearing on the interpretation of the effects of vagotomy.

METHODS. In all experiments cats were used, either decerebrated under ether at a level just above the superior colliculi, or lightly anesthetized with chloralose and urethane (40 mgm. and 0.4 gm. per kgm. respectively), supplemented with ether during the preparatory operations. On two occasions Dial was used intraperitoneally. After tracheotomy, isolation of the vagi and (when blood pressure was to be recorded) cannulation of one carotid, the animals were placed in a body plethysmograph. Air-tight closure around the base of the neck was secured with plaster of Paris. An outlet from the plethysmograph was connected, as illustrated in figure 1, with a 20-liter bottle and with a mercury manometer by which the degree of inflating or deflating force was controlled. Sometimes the manometer was arranged to record the inflating or deflating pressure. The purpose of the large bottle was to minimize the changes of pressure in the plethysmograph during the animals' respiratory movements. Breathing was recorded by a spirometer of the Krogh type, connected through a soda lime cannister to the tracheal cannula. Oxygen was bubbled into the closed system just fast enough to replace that consumed. This arrangement allows the quantitative measurement of *a*,

the tidal air; *b*, the immediate change in lung volume on vagotomy; *c*, the change in lung volume on inflation or deflation; *d*, the resistance to inflation and deflation, which can be expressed as the ratio of applied pressure. to the change in lung volume.

In the decerebrate animals, both carotids were tied, thus securing uniform stimulation of the receptors of the carotid sinus region before and after vagotomy. The possibility of pressure changes in the aortic region affecting the results is not eliminated, nor is the effect of changes of blood pressure acting on the medulla.
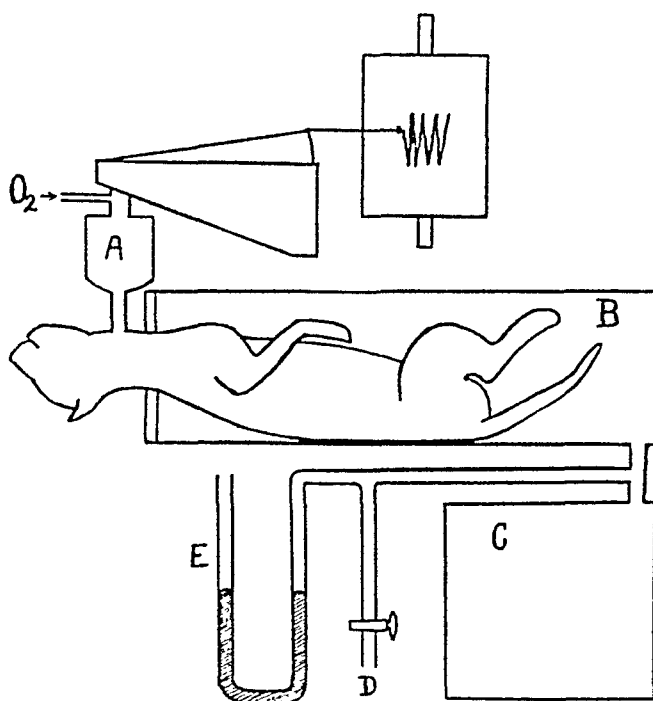
Fig. 1. *A* is a cannister containing soda lime connecting a tracheal cannula in the cat with the spirometer; *B* is the body plethysmograph; *C*, a twenty-liter bottle; *D*, a side-arm for the application of suction or pressure; *E*, a manometer.

RESULTS. The results most typical in this series will be presented first. Interesting but irregular results will be dealt with for the most part in the discussion.

Typical effects of inflation and deflation before and after vagotomy are shown in figures 2 and 3. With sufficient inflation there was as a rule a pause in the breathing, during which a progressively mounting expiratory effort was very evident on the tracing. This was terminated abruptly by a vigorous inspiratory effort. The cycle then repeated itself with increasing frequency, in consequence no doubt of the inadequate pulmonary ventilation.

On deflation of the chest there was in most experiments (12 out of 15) a

prompt increase in frequency. In the most sensitive of the animals a decrease of 5 cc. in a cat weighing 2.2 kgm. was sufficient to double the respiratory rate.
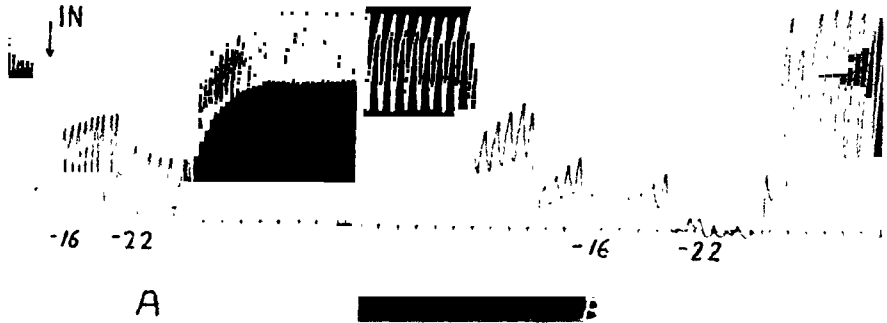
Fig. 2. Showing the respiration and effects of inflation of the chest of cat C (decerebrate), A about five minutes before and B about five minutes after double vagotomy. −16 and −22 refer to the negative pressure in the plethysmograph in millimeters of mercury. Note the greater lung volume during distention with the same negative pressure after vagotomy. Time interval, 5 sec.
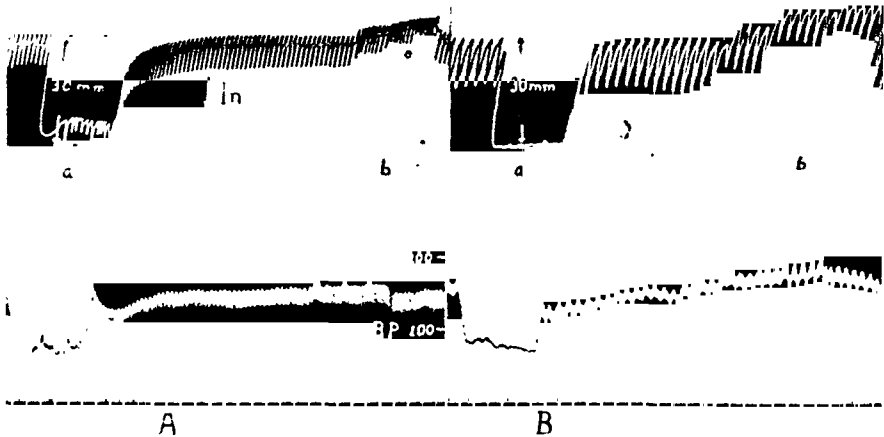
Fig. 3. Tracing from cat B (decerebrate), shows in addition, the effects of deflation of the chest and a record of blood pressure. A was taken about three minutes before and B about three minutes after vagotomy. Little a marks the period of application of a negative pressure of −22 mm. Hg., both before and after vagotomy. Little b marks compression of the chest by successive application of 5, 10, and 15 mm. Hg positive pressure both before and after vagotomy.

After vagotomy the following changes are demonstrated in figures 2 and 3. 1. The lung volume at the end of expiration is unchanged (fig. 5). (This was true in nine out of ten experiments.) 2. The respiratory rate is slower. Only two cats which were rather deeply anesthetized failed to

show this change.   3. No pause in breathing follows inflation.   4. The shape of the tracing during inflation suggests decreased expiratory effort. 5. The resistance to inflation is less.   6. On deflation no increase in frequency is evident, nor did it occur in any experiment.   7. The resistance to deflation is also less.   8. The speed of inspiration within two minutes after vagotomy is definitely slower than before vagotomy (fig. 4).   This effect was observed in four out of eight experiments; in the other four no definite change was demonstrable.

The observation that vagotomy often decreases the speed of inspiration substantially confirms the findings of Gesell, Steffenson and Brookhart (1937).   Hammouda and Wilson, however, report that immediately after vagal section the inspirations are deeper and faster, only settling
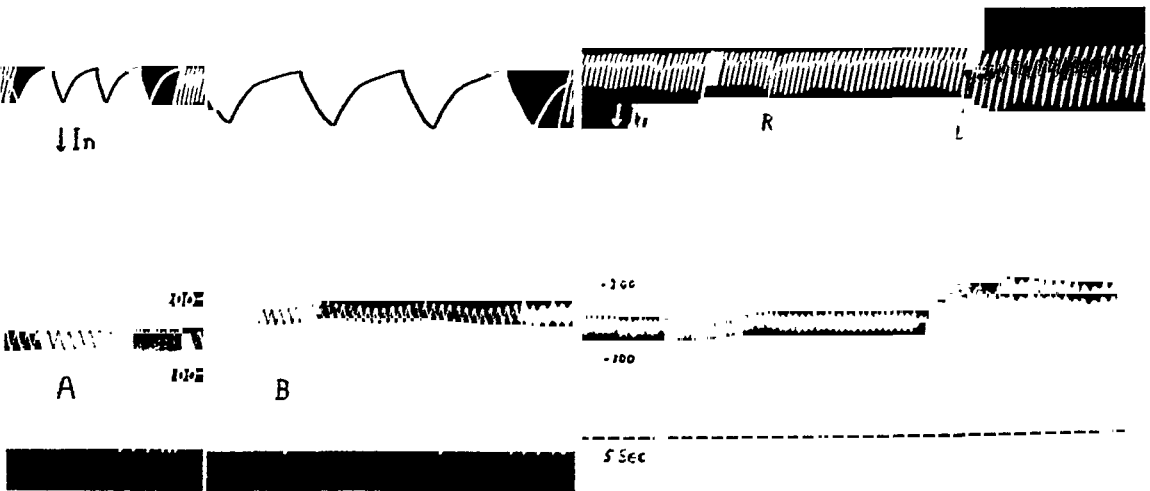
Fig. 4   Fig. 5

Fig. 4. A faster record showing a decreased speed of inspiration (less slope) after vagotomy.   A two minutes before, B two minutes after vagotomy.

Fig. 5. Shows the constancy of lung volume at the end of expiration of cat B after cutting the right vagus, R, and the left vagus, L, in the neck.

down to their previous speed after about half an hour.   It seemed important to determine the part played by the level of the chemical stumulus after vagotomy in these variable results.

Blood gases after vagotomy.   Five animals were used, three decerebrate and two anesthetized with chloralose and urethane.   They were prepared as in other experiments by tracheotomy, isolation of the vagi and cannulation of one carotid artery.   Four samples of arterial blood were taken at twenty to twenty-five minute intervals, two before vagotomy and two after, and analyzed for carbon dioxide and oxygen in a Van Slyke apparatus.   In every instance the arterial CO_2 content was higher ten minutes after vagotomy than it was ten minutes before vagotomy.   The smallest rise was 0.3 v.p.c., the largest 2.5 v.p.c.   Consequently it may

be assumed that the slower speed of inspiration after vagotomy could hardly have been due to a lower arterial CO_2 content. The cases in which no definite change in the speed of inspiration could be detected may well have had sufficient rise in arterial CO_2 to offset the tendency of vagotomy to decrease the speed of inspiration. In the second blood sample taken after vagotomy (i.e., one-half to one hour after vagotomy) the CO_2 had, in all cases but one, fallen. It is hard to say, without further experiment,

TABLE 1

Effects of vagotomy on the gases of the arterial blood

	TIME IN MINUTES				
	0	29	42	55	79
Cat 1, anesthetized					
CO_2 v.p.c.	29.2	29.5	Vagotomy	32.1	33.5
O_2 v.p.c.	18.3	17.5	Vagotomy	18.2	17.7
	0	25	34	47	65
Cat 2, decerebrate					
CO_2	30.6	28.7	Vagotomy	29.5	22.5
O_2	18.2	15.8	Vagotomy	14.8	14.5
	0	30	40	50	65
Cat 3, decerebrate					
CO_2	27.0	23.9	Vagotomy	25.6	25.1
O_2	17.2	16.2	Vagotomy	15.7	15.7
	0	22	27	44	99
Cat 4, decerebrate					
CO_2	36.0	37.7	Vagotomy	38.9	35.0
O_2	15.6	15.1	Vagotomy	15.4	14.7
	0	28	40	50	74
Cat 5, anesthetized					
CO_2	44.8	43.7	Vagotomy	44.0	42.1
O_2	16.2	15.7	Vagotomy	15.7	15.4

whether this delayed fall in arterial CO_2 is due to overventilation or to circulatory or metabolic effects of vagotomy. The O_2 content of the blood declined slightly in all the series, the last sample containing 1 to 3 v.p.c. less oxygen than the first.

The great increase in speed and depth of inspiration after vagotomy in Hammouda and Wilson's experiments may have been due to the use of morphine, since according to Henderson and Rice (1939) one of the effects of morphine is to augment the effectiveness of the vagal impulses

inhibitory to inspiration. The relation of changes of blood pressure to these results will be considered later.

Resistance to inflation and deflation. In figures 2 and 3 are shown the changes in lung volume produced by equal pressure changes before and after vagotomy. After vagotomy the changes in lung volume to both inflating and deflating pressures are definitely greater. It is difficult to interpret these effects otherwise than as due to removal of active resistance to deflation (by inspiratory muscles) and active resistance to inflation (by expiratory muscles). For example, it is difficult to see how increased inspiratory activity due to release of inhibition by the vagi could possibly explain a decreased resistance to deflation, as well as to inflation. Nor would the invocation of decreased inhibition to both inspiration and expiration explain the facts any better. Speaking strongly for an augmentor influence on expiration transmitted by the vagi during inflation, is the greater amplitude of breathing during inflation before vagotomy, whereas at normal lung volume the amplitude is greater after vagotomy.

After pithing the brain and cord there is a still further slight decrease in resistance to inflation, but not to deflation. In addition, there is a loss of elasticity of the chest in the sense that the chest does not quite return to its original volume after distortion. Apparently after complete denervation the chest is, within certain narrow limits (e.g., 25 cc. in a 3 kgm. cat) plastic. It stays at the volume at which it is set.

DISCUSSION. The reactions of the completely denervated chest raise a question as to the propriety of the term "passive expiration." It seems hardly proper to apply the term passive to an expiration in which tonically active muscles participate. And it seems even less proper to apply it if the tonically active muscles have been reciprocally inhibited during inspiration. Perhaps an even more important reason for abandoning the term "passive expiration" is that while it is easy enough to distinguish expirations of varying degrees of activity, it is a practical impossibility to determine that any expiration, is purely passive, i.e., unassociated with any increased activity in any expiratory muscle.

Hammouda and Wilson (1932) conclude that "there is no evidence of an immediate active expiratory response to expansion or an inspiratory response to collapse implied in the Hering-Breuer hypothesis." This statement is perhaps understandable in view of these authors' preoccupation with the phenomena of increased frequency on deflation and decreased frequency on inflation, but can hardly be accepted. Examination of their own tracings for changes of resistance to inflation on vagotomy gives the evidence which they have overlooked. Even casual inspection of their tracings suggests a definite difference in the character of expiration during inflation, before and after vagotomy. They appear to have been misled by two conditions of their experiments. 1. The use of progressively increasing pressures for expansion and compression, instead of steady

pressures. 2. The occurrence of definite changes in lung volume on vagotomy in their published tracings.

Changes in lung volume on vagotomy. In this series in only one experiment out of ten was there a change in lung volume after vagotomy which lasted more than one minute. In that experiment the lung volume at the end of expiration was increased for an indefinite period. In most of the other experiments only a transitory increase or decrease lasting for only two or three breaths was observed. The tracings of Hammouda and Wilson show in one case a considerable increase, in the other a considerable decrease after vagotomy. Head (1889) describes prolonged tonic contraction of the diaphragm after vagotomy as typical, but not invariable. The state of the chest or degree of expansion of the lung immediately prior to section is not always specified and must play an important part (Hess, 1936). The fact that no change of lung volume occurred on vagotomy in most of these experiments must mean that either no change occurred in tonus of the respiratory muscles or that changes of equal effectiveness occurred in the expiratory and inspiratory muscles. This is important in the interpretation of the larger lung volume at the end of expiration during inflation after vagotomy, which might be attributed to decreased activity of expiratory muscles or to relatively increased activity of inspiratory muscles at the end of expiration. The latter explanation is incompatible with the finding of unchanged lung volume at the end of expiration when under no distorting pressure.

It is not suggested that active expiration is entirely dependent on afferent vagal impulses. Adrian (1933) has noted its presence after vagotomy, as evidenced by action currents in expiratory muscles. Evidence to the same effect has been obtained in experiments in this series by attaching a Cushny recorder to the rectus abdominis muscle. Rhythmic inhibition and activity were not easily detected during quiet breathing but were very evident during asphyxia. Similar expiratory activity was observed during asphyxia after vagotomy. There was, however, a very definite delay after the cessation of inspiration before the onset of the expiratory contraction as contrasted with an immediate sequence of expiration after inspiration before vagotomy.

In view of the suggestion of Hammouda and Wilson that the increased frequency of breathing on deflation was due to tonic impulses augmentor to frequency acting on a pneumotoxic centre when these impulses were opposed by fewer inhibitory impulses generated by stretching of the lungs, it seems important to point out that the increased frequency is not an invariable response to deflation, even when the vagal reflexes are not depressed at all by anesthesia. Sometimes slowing occurs, or slowing followed for no apparent reason by increased frequency or vice versa. In one case mild deflation produced inspiratory spasms or apneuses.

Adrian (1933) has supported very plausibly the hypothesis that most

of the phenomena attributed to afferent vagal impulses might be explained by postulating only impulses inhibitory to inspiration and evoked by inflation of the lungs. It is probably futile to attempt to distinguish between impulses directly inhibitory and reciprocally inhibitory but it seems important to reiterate that the experiments reported in this paper allow the inference of impulses directly augmentor to inspiration and expiration (and hence of reciprocal inhibition) with more certainty than the inference of independent inhibitory action.

Schmidt (1932) has shown that changes in blood pressure can cause changes in respiration by their effects on blood flow through the medulla as well as by their effects on receptors in the aortic and carotid sinus regions. Vagotomy in these decerebrated animals has usually resulted in a considerable rise in blood pressure (30 or 40 mm. Hg). The effect of such a rise would be to depress respiration even after denervation of the carotid and aortic arch receptors, and might be an important factor in explaining the decreased velocity of inspiration after vagotomy. Till this factor is properly controlled the existence of the proprioceptive impulses augmentor to inspiration postulated by Gesell, Steffanson and Brookhart to arise during inflation must remain in question.

Inflation of the chest in our experiments caused a large fall in blood pressure (fig. 3), while deflation caused a smaller rise. It seems unlikely that on deflation the rise in blood pressure (since it is in general terms depressant to respiration) could be in any part responsible for the greater inspiratory activity evident while the vagi were intact. On the other hand, it seems quite possible that during inflation the fall in blood pressure might well be in part responsible for the greater expiratory activity observed while the vagi were intact.

SUMMARY

The effects on respiration of inflation and deflation of the lungs in decerebrate and anesthetized cats have been recorded mechanically, before and after vagotomy. The changes after vagotomy include 1, decreased resistance to inflation; 2, decreased resistance to deflation; 3, decreased speed of inspiration.

Vagotomy results in a small rise in arterial CO_2 followed in about an hour by a fall.

The probable effects of changes in blood pressure are discussed in relation to the results as a whole.

The results are interpreted as evidence of the importance of the afferent influences of the vagi, postulated by Hering and Breuer to be augmentor to expiration on inflation and augmentor to inspiration on deflation.

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CEREBROSPINAL FLUID PRESSURE AND VITAMIN A DEFICIENCY[1]

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In previous publications Moore, Huffman and Duncan (1935) and Moore (1939a, 1939b) reported a type of blindness resulting from a constriction of the optic nerve as due to a deficiency of vitamin A in the ration of the bovine. Blindness was preceded by papilledema, nyctalopia, incoördination and syncope and a decrease in the carotene content of the blood plasma. In private communications several investigators have since reported papilledema in calves fed vitamin A deficient rations. The presence of papilledema is usually considered prima facie evidence of an elevated cerebrospinal fluid pressure. Since some of the manifestations accompanying this syndrome, particularly the papilledema, syncope, and incoordination, might be accounted for by the presence of an increased cerebrospinal fluid pressure, pressures were determined on young bovine fed a vitamin A deficient ration and are reported in this paper.

EXPERIMENTAL. Blood plasma carotene determinations were made each week according to a recently published method by Moore (1939c). For the carotene determinations of the alfalfa meal, the method of Guilbert as modified by Peterson et al. (1937), was used.

The low carotene ration consisted of skimmed milk and a concentrate mixture consisting of 240 pounds barley, 180 pounds rolled oats, 180 pounds wheat bran, 60 pounds linseed oil meal and 8 pounds salt. This ration contained sufficient carotene to supply two to four micrograms per kilogram of body weight per day. Wood shavings were used as bedding.

Cerebrospinal fluid pressures were obtained by puncture into the subarachnoid space. The insertion into the subarachnoid space was made through the dorsal opening in the atlanto-occipital articulation. No anesthetic was used and the records were obtained with the animals in the standing position.

The animals used were of the Holstein breed and were for the most part the same individuals described in previous reports by Moore (1939a, 1939b). The principal plan was to limit the carotene intake till a change in cerebrospinal fluid pressure occurred and then to add a supplement in

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[1] Journal article 421 (n.s.) Michigan Agricultural Experiment Station.

an attempt to restore the pressure to normal.   At the same time ophthalmoscopic observations were made at appropriate times and the animals were tested for night blindness by attempting to run them into objects and observing their behavior in dim light, a method similar to that used by Guilbert and Hart (1935).   The animals were weighed every 10 days at which time adjustments were made in the amount of carotene supplement fed.   Data on only three of the experimental animals are presented. These data are representative of the group as a whole.

Animal 1 was a Holstein male which had been receiving since 179 days of age a carotene supplement in the form of alfalfa leaf meal supplying 60 micrograms of carotene per kilogram of body weight per day.   At 598 days the supplement was removed from the ration.   At this time the animal showed no fundus changes and appeared normal and active.   The cerebrospinal fluid pressure was also normal as shown in graph I.   At 663 days this animal showed night blindness and the cerebrospinal fluid pressure rose from a normal of 110 mm. of saline to 200 mm.   A slight edema of the nerve head developed at 709 days of age at which time pressure had increased to between 250 and 300 mm. of saline.   At 720 days the tapetum lucidum was quite bleached of its normal yellow color and there was slight edema at the edges of the nerve head.   A small hemorrhage was noticed on the nerve head of each eye and the capillaries were distended.   The calf showed diarrhea, some incoördination and swelling at the hocks.   At 721 days alfalfa supplying 120 micrograms of carotene per kilogram of body weight was added to the ration.   The cerebrospinal fluid pressure decreased, plasma carotene increased and the night blindness disappeared.   However, the cerebrospinal fluid pressure did not return to normal even after 109 days of supplemental feeding although the slight edema of the nerve head had almost disappeared.   At this time the animal had recovered from the effects of the deficiency as judged by external appearance and was disposed of because he was too large to manage effectively.   At autopsy no gross changes were noted.

Animal 2 had received crystalline carotene dissolved in cottonseed oil since 109 days of age at the rate of 300 micrograms per kilogram of body weight.   At 178 days the level was reduced to 120 micrograms.   At 498 days of age when the animal appeared in normal health and no fundus changes could be noticed the carotene supplement was removed from the ration.   Nyctalopia and papilledema developed at 536 days.   At 563 days the cerebrospinal fluid pressure had increased from 115 mm. to 250 mm. of saline and both eyes showed a choking of 3 diopters.   At 568 days the supplement was again added to the ration.   Night blindness disappeared in about 6 days, and the plasma carotene showed an immediate increase. The cerebrospinal fluid pressure also dropped but remained above normal. Graph II shows the changes which occurred in this calf.   It was necessary
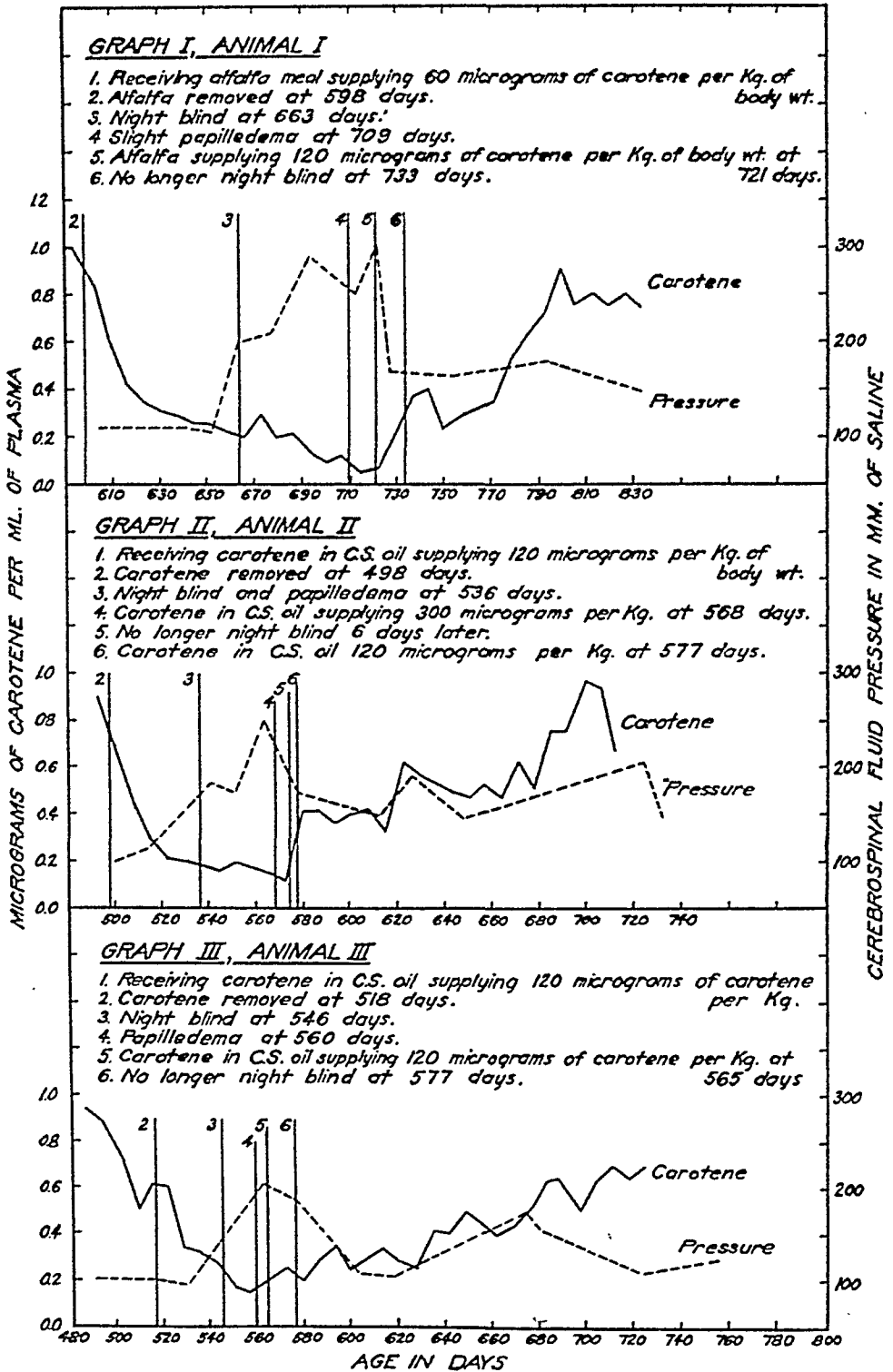
Fig. 1

to dispose of the animal at 733 days of age. At this time the papilledema had decreased to about 2 diopters, and its presence was confirmed at autopsy. The ventricles of the brain were also considerably distended being 3 to 4 times their normal size and the choroid plexus appeared edematous.

Animal 3 had been receiving crystalline carotene dissolved in cottonseed oil at the rate of 120 micrograms of carotene per kilogram of body weight since 188 days of age. At 518 days of age when the animal was apparently in good health and showed no ocular changes the crystalline carotene was removed from the ration. The cerebrospinal fluid pressure at this time was 100 mm. At 546 days as shown in graph III this animal was night blind and at 560 days showed edema of the nerve head, bleaching of the tapetum lucidum, considerable exophthalmus and some incoördination. At this time the cerebrospinal fluid pressure had increased to 200 mm. of saline. At 565 days the crystalline carotene supplement was added to the ration. When examined with the ophthalmoscope at 572 days the right eye showed a choking of 3 diopters and the nerve margins were blurred. There were about 10 petechial hemorrhages on the nerve head. These hemorrhages had largely disappeared a week later or after about 2 weeks of supplemental feeding. At 577 days the animal was no longer night blind. The cerebrospinal fluid pressure decreased to normal but later showed two higher values and then again returned to the normal level. The edema of the nerve head gradually decreased also.

DISCUSSION. The evidence presented indicates that a deficiency of vitamin A in the ration of the bovine permits an elevated cerebrospinal fluid pressure to develop. The results on all the animals show definitely' that vitamin A deficiency and a low plasma carotene is correlated with a raised cerebrospinal fluid pressure. When vitamin A was withdrawn from the ration plasma carotene decreased and the pressure increased. When the deficiency was corrected the plasma carotene increased and the cerebrospinal fluid pressure decreased. The results with animals 2 and 3 afford almost indisputable proof of this relationship since the effect was produced by removing crystalline carotene from the ration and was alleviated by supplementing the ration with carotene. It will be noted that in only one instance (animal 3) did the cerebrospinal fluid pressure completely return to the normal level. Apparently the disturbances which are produced by vitamin A deficiency are slow in repair. It would appear to be a matter of 4 or 5 months. Unpublished observations on mature animals in studying the rate of decrease of papilledema also indicate a long period of recovery even when the animals are turned to pasture.

It will be noted that there were some variations in the time of first appearance and amount of papilledema and the development of cerebrospinal fluid pressure. Animal 1 showed a very high cerebrospinal pressure

and yet never developed a marked papilledema. These variations seemed to be more pronounced as the animals grew older. In unpublished observations on mature cows depleted of vitamin A, incoördination and syncope were present without marked fundus changes. If left on the ration sufficiently long, however, in most cases the fundus changes would finally occur. One would conclude, therefore, that the papilledema is much more easily produced in young animals. These differences might be explained by changes in intraocular tension. Studies should be made on mature animals concerning the relationship of the development of increased cerebrospinal fluid pressure and fundus changes. However, considerable difficulty is encountered in making repeated pressure measurements with larger animals.

In general the data on these calves show a rather definite correlation between papilledema and an elevated pressure, and it would seem reasonable to suggest that the papilledema is a direct result of the elevated cerebrospinal fluid pressure. It would also be possible to explain the papilledema as due to pressure on the optic nerve caused by stenosis of the optic canal, a condition which develops if the deficiency persists for long periods (Moore, 1939a, b). That the latter is not the true explanation, is indicated by the fact that papilledema occurs in mature cows due to vitamin A deficiency without any apparent stenosis of the optic canal.

In two of the calves (1 and 3) incoördination developed when the cerebrospinal fluid pressure had increased considerably and when the fundus changes were most pronounced. The incoördination rapidly disappeared when vitamin A was added to the ration coincident with the fall in pressure. This likewise suggests that the incoördination observed in vitamin A deficiency in the bovine may be related to cerebrospinal fluid pressure changes. On the other hand, the incoördination may be due to myelin degeneration. This was not determined. However, in pigs incoördination in vitamin A deficiency may not be correlated with myelin degeneration according to work reported by Eveleth and Biester (1937) and Bessey and Wolbach (1938) have expressed some doubt as to a relationship between myelin degeneration and vitamin A deficiency. One would not suspect that a condition of syncope (Moore, 1939a) was associated with myelin degeneration.

The question as to whether vitamin A deficiency produces similar changes in other species is interesting. Preliminary data with dogs indicate fairly conclusively that increased cerebrospinal fluid pressure develops on vitamin A deficient rations but that the fundus changes do not readily occur. Mellanby (1938) has noted widespread nerve degeneration in young dogs fed diets deficient in vitamin A and rich in cereals. Complete deafness was present in some of the animals which he attributed to nerve degeneration due to an overgrowth of bone of the labyrinthine cap-

sule. ' He expressed the belief that the degeneration of other nerves might be due to pressure caused by overgrowth of bone in the various foramina. It is suggested that the changes reported by Mellanby (1938) are related in some manner to those reported in calves where a constriction of the optic nerve develops.

CONCLUSIONS

1. A deficiency of vitamin A in the ration of the young bovine produces an increased cerebrospinal fluid pressure.

2. The increase in cerebrospinal fluid pressure is accompanied by papilledema, nyctalopia, syncope and incoördination.

3. On return to a normal diet the cerebrospinal pressure slowly returns to normal, while the quoted disturbances disappear.

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THE RECEPTIVE FIELDS OF OPTIC NERVE FIBERS

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Appreciation of the form of the retinal image depends upon a correspondence between the distribution of light on the retina and the distribution of activity among the fibers of the optic nerve. This correspondence may be studied directly by recording the activity in single optic nerve fibers in response to illuminating various parts of the retina.

A given optic nerve fiber responds to light only if a particular region of the retina receives illumination. This region is termed the receptive field of that fiber. In a previous paper describing the responses in single optic nerve fibers from the cold-blooded vertebrate eye (Hartline, 1938) it was noted that the receptive fields of the optic nerve fibers are of small but appreciable extent, and that their locations on the retina are fixed. It is the purpose of the present paper to describe further the characteristics of receptive fields, and to discuss some of the spatial factors involved in the excitation of the fibers of the optic nerve.

METHOD. The method for recording the activity in single optic nerve fibers from the eyes of cold-blooded vertebrates has been described in the previous paper (loc. cit.). An eye is excised, cut open, and small bundles of optic nerve fibers are dissected from the anterior surface of the exposed retina. The action potentials in these bundles are amplified and recorded with an oscillograph. When such a bundle has been split successfully, until only a single active fiber remains, the retina must be searched with a small spot of light to determine the region supplying that fiber. This search is aided by noting the direction, on the retina, from which the nerve fibers in the small bundle come, and by using large spots of light at first to locate the approximate position of the sensitive region.

The optical system employed in these experiments has likewise been described. A spot of light of suitable size is projected upon the exposed retina; the coördinates of its position, referred to an arbitrary point of origin on the retina, are obtained from readings of crossed micrometers which control its location. The micrometer readings are reduced to millimeters on the retina by multiplying them by the magnification of the optical system (0.32 or 0.15). Sharpness of focus of the spot on the retina

is checked in every experiment by direct observation through a dissecting microscope.[1] This optical system can provide a maximum intensity of illumination on the retina of $2 \cdot 10^4$ meter candles, which may be reduced to any desired value by means of Wratten "Neutral Tint" filters.

Eyes from large frogs (R. catesbiana), and from a few alligators, were used in the present study. In none of these experiments did the receptive fields of the fibers lie in or near the *area acuta* of the retina; this paper is therefore concerned only with properties of the peripheral retina. The preparations were always allowed 20 to 30 minutes for dark adaptation (at 25°C.), and observations were checked whenever possible to guard against slow changes in sensitivity.

RESULTS. The sensitivity of different regions of the retina to light must be defined with respect to the particular optic nerve fiber which is under observation. A spot of light in one location on the retina may elicit a vigorous discharge of impulses in an optic nerve fiber, but in a different location may produce no responses at all in this particular fiber. The distribution of sensitivity over the receptive field of a fiber may be determined by systematic exploration with a small spot of light, noting the responses elicited at various locations, and charting the boundaries of the region over which the spot is effective, at different intensities.

In figure 1 are given two examples. Figure 1a was obtained with a fiber whose responses consisted of a burst of impulses when the light was turned on, and another burst upon turning it off.[2] At the highest intensity (log I = 0.0) the exploring spot (50 μ in diameter) would elicit responses if located anywhere within the outermost closed curve. The

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[1] Although sharply focussed, such a spot of light on the retina is always surrounded by a faint halo of scattered light. This is due chiefly to Tyndall scattering in the layers of the retina overlying the rods and cones (diffraction, and reflection and scatter from the surfaces of the optical system contribute only a small amount). The relative intensity of this halo has been estimated by direct observation in several fresh preparations. A piece of gelatin neutral-tint filter was placed in the eye-piece of the dissecting microscope, just covering the image of the spot of light on the retina. With a large spot of light (1 mm. square), filters of densities between 2.0 and 3.0 were necessary to reduce the intensity of the spot, seen through the filter, to match approximately the intensity of the halo of scattered light, seen over the edge of the filter. Thus in nearly all cases the intensity of the halo, within a few microns of the edge of the spot, is 1 per cent or less of the spot intensity, and falls off rapidly with increasing distance from the edge of the spot.

[2] It has been shown previously that different optic nerve fibers of the vertebrate eye give different kinds of discharges in response to illumination of the retina. In some of the fibers impulses are discharged steadily as long as the light shines; others give only a brief burst of impulses when the light is turned on, and again when it is turned off; still others respond only to turning the light off. The general characteristics of the receptive fields of different fibers, however, are essentially the same, regardless of their type of response.

dots mark locations at which the spot could just elicit a response, at this intensity. For such locations on the boundary, both the "on" and the "off" bursts consisted of only one or two impulses, but locations inside the boundary gave rise to stronger discharges, and when the spot was located in the center of the region, vigorous bursts were obtained. At a lower intensity (1/100 of the former: log I = −2.0) responses could be obtained only when the spot was located within the much smaller region enclosed by the innermost curve, and at this intensity the discharges
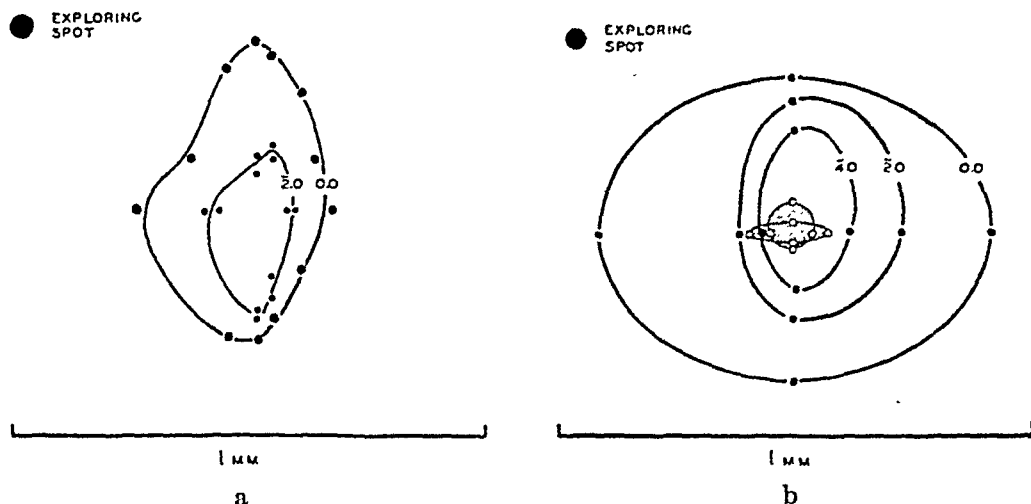
Fig. 1. Charts of the retinal regions supplying single optic nerve fibers (eye of the frog). a. Determination of the contours of the receptive field of a fiber at two levels of intensity of exploring spot. Dots mark positions at which exploring spot (50 μ diameter) would just elicit discharges of impulses, at the intensity whose logarithm is given on the respective curve (unit intensity = 2.10⁴ meter candles). No responses at log I = −3.0, for any location of exploring spot. This fiber responded only at "on" and "off." b. Contours (determined by four points on perpendicular diameters) of receptive field of a fiber, at three levels of intensity (value of log I given on respective contours). In this fiber steady illumination (log I = 0.0 and −2.0) produced a maintained discharge of impulses for locations of exploring spot within central shaded area; elsewhere discharge subsided in 1–2 seconds. No maintained discharge in response to intensities less than log I = −2.0; no responses at all to an intensity log I = −4.6.

were very weak even when the spot was located in the center of the region. At a tenth of this intensity, log I = −3.0, no responses could be obtained for any location of the exploring spot whatever.

Figure 1b is a chart of the receptive field of another fiber, which in this case was capable of a steady discharge of impulses, maintained as long as illumination lasted. As in the previous experiment, the brighter the exploring spot, the larger was the region over which it would elicit responses, and, at any given intensity, the responses were stronger the more nearly central the location of the exploring spot. Indeed, it was

only for locations in the very center (cross-hatched region in fig. 1b) that the discharge would be maintained throughout an indefinitely long period of illumination. Elsewhere it would subside and finally stop in a second or two (cf. Hartline, loc. cit., fig. 6). At the lowest intensity represented in the figure (log I = −4.0) no maintained discharge could be obtained at all; the responses consisted of only a few impulses, and at $\frac{1}{4}$ of this intensity (log I = −4.6) no responses whatever could be elicited.

These experiments show that the sensitivity to light, referred to a particular optic nerve fiber, is not uniform over the fiber's receptive field. The central portion of the receptive field has a lower threshold and, at intensities above threshold, gives rise to stronger responses than the outlying areas. The sensitivity is thus maximal in the center, and falls off steadily with increasing distance from this center, to become inappreciable outside an area approximately one millimeter in diameter. Charts such as those of figure 1 are contour maps of this distribution of sensitivity. The faint halo of scattered light surrounding the exploring spot is a source of error in the construction of these charts. However, at relatively low intensities (100 or even 1000 times the minimum threshold) this scattered light is of little consequence, and a map obtained at these intensities must closely approximate the actual distribution of sensitivity over the receptive field of the fiber under observation.

Factors other than the absolute intensity of the exploring spot affect the extent of the region from which responses in a given fiber can be elicited. If the exploring spot is made smaller, its intensity must be increased if it is to be effective over as large an area. But with this smaller spot the threshold measured in the most sensitive central region is correspondingly increased. It is the intensity relative to this minimum threshold which is significant in charting the distribution of sensitivity. Similarly, if the retina is not completely dark adapted, its level of sensitivity is decreased, and for a particular fiber the thresholds in the center and on all the contours of its receptive field are increased proportionately. Receptive fields of different fibers must likewise be compared with due regard to their minimum thresholds, which may differ considerably.

The vertebrate retina responds vigorously to small, sudden movements of the retinal image (Ishihara, 1904; Adrian and Matthews, 1927). This may be observed in the responses of single optic nerve fibers, and is helpful in determining the distribution of sensitivity in their receptive fields. Figure 2 shows records of the discharge in a fiber responding at "on" and "off." Although no impulses were discharged while the spot of light was shining steadily, a slight movement of it, of only a few microns in any direction, produced a short burst of impulses. Responses to movement are stronger, within limits, the larger and more intense the moving spot, and the greater and the more rapid its displacement. Responses to a

slight movement of a spot of light of given size and intensity can be elicited anywhere within the region over which this spot is effective in producing discharges when it is turned on or off. They are weak when the spot is near the boundary of this region, and stronger the more nearly central its location in the receptive field. Figure 3 shows the contour
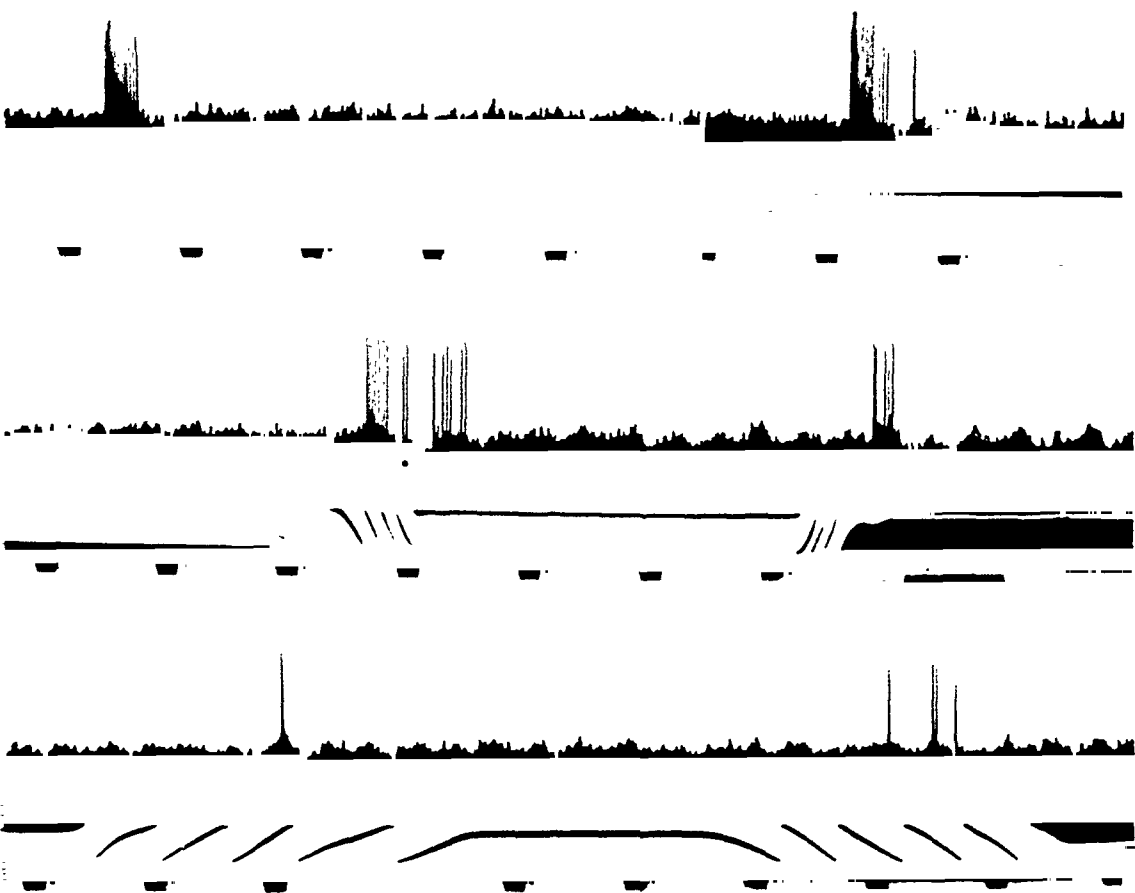
Fig. 2. Oscillograms of action potentials in a single optic nerve fiber (frog), showing responses to slight movements of small spot of light (50 μ diameter) on the retina. Fiber responded only at "on" and "off"; no discharge during steady illumination if stimulus spot was stationary (upper record; signal marking period of illumination blackens the white strip above time marker). Slight movements of stimulus spot elicited short bursts of impulses (middle and lower records). Movements of spot on retina are signalled by narrow white lines appearing above time marker; these are shadows of spokes attached to head of micrometer screw controlling position of stimulus spot. Each spoke corresponds to 7 μ on the retina. Time in ⅕ second.

within which a spot of light 50 μ in diameter, about 100 times the minimum threshold, produced responses in a fiber responding to "off" only. The arrows show the limits, on two diameters, between which slight movements of this spot (ca. 20 μ in ca. 0.05 sec.) would elicit bursts of impulses. Outside of these limits no responses to movement could be

obtained, no matter how great or how rapid the displacements. It is characteristic of a fiber which responds only to "off" that it also responds only to movements of the spot away from the center of its receptive field.

Bursts of impulses are also elicited in response to movement of a shadow on the uniformly illuminated retina. A slight, sudden movement of a narrow band of shadow produces responses if it falls across the receptive field of the fiber under observation, and these responses can be elicited over a region many times wider than the shadow itself. To show this, all diaphragms were removed from the optical system, and a fine wire
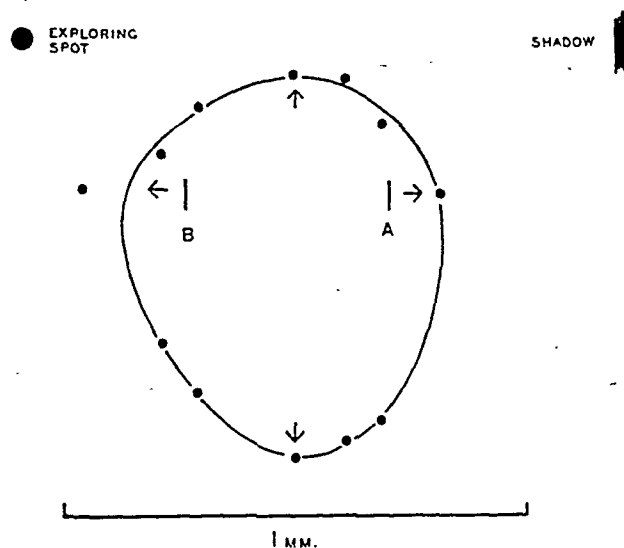
Fig. 3. Chart of the receptive field of an optic nerve fiber (frog), showing limits within which responses were elicited by movements of an illuminated spot, and of a narrow band of shadow. Dots mark locations at which exploring spot produced responses when turned off (fiber responded only to "off"). Spot 50 μ in diameter, intensity 100 times minimum threshold. Arrows mark the limits (on two diameters) between which slight movements of illuminated spot elicited bursts of impulses. With large area of retina illuminated (4 mm. diameter) a band of shadow 20 μ wide produced discharges of impulses when moved slightly, if it crossed the receptive field within the limits marked by the vertical lines A and B. Shadow extended across entire illuminated area, in direction lengthwise of page; movements were crosswise. See figure 4 for records of responses to moving shadow.

was stretched across the beam. This yielded a circular patch of light on the retina, 4 mm. in diameter, across which was a band of shadow 20 μ wide. In the experiment of figure 3 the limits within which slight movements of this shadow produced responses are indicated by the vertical lines, A and B. If the shadow was near either of these limits the responses to its movement were weak, as shown in the upper and the lower records of figure 4, while if it fell across the center of the sensitive region the same amount of displacement elicited stronger bursts of impulses (middle record of fig. 4). Responses to movement of a shadow are elicited regardless of

the direction of the motion, both in the fibers responding to "on" and "off" and in those responding to "off" only.

From these experiments it is evident that the receptive field of an optic nerve fiber from the peripheral retina covers an area much greater than
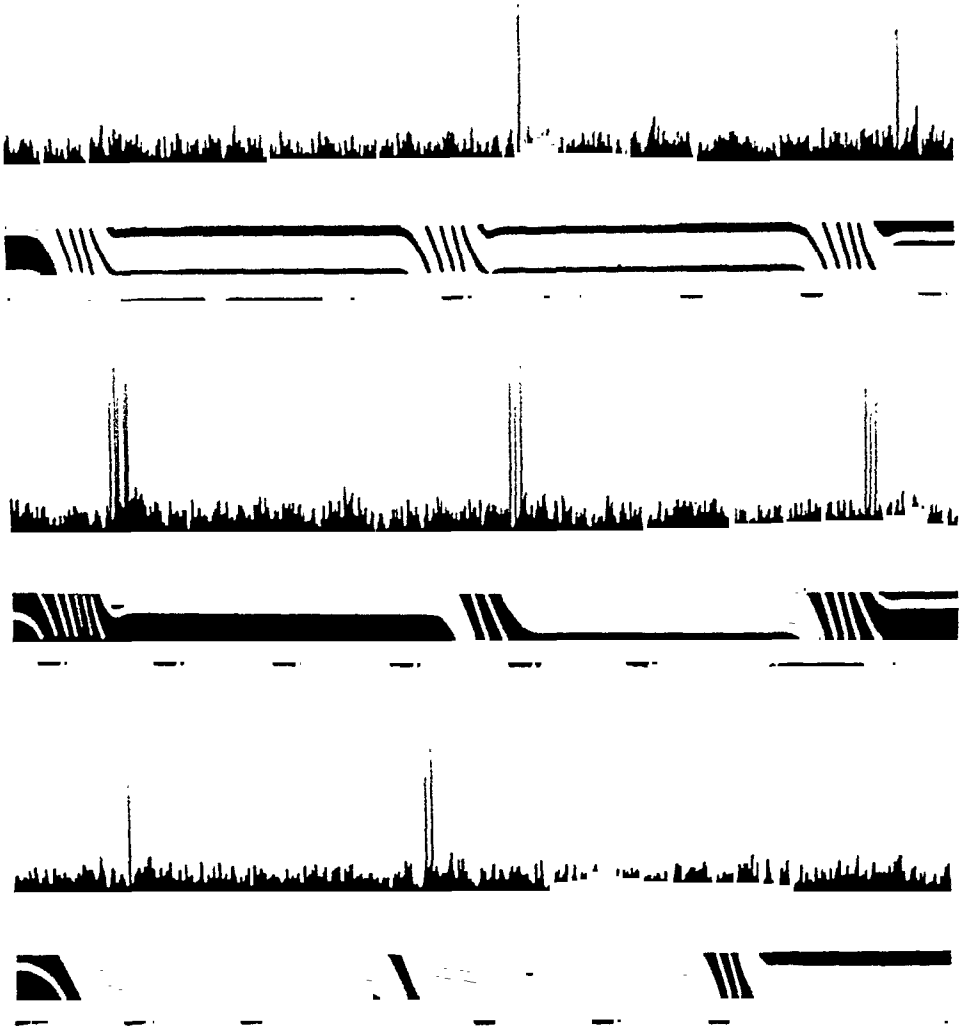
Fig. 4. Records of the impulses discharged in an optic nerve fiber in response to movement of a shadow on the retina. Experiment of figure 3. Narrow band of shadow, on uniformly illuminated retina, was moved from right to left (chart of fig. 3) in a series of short, quick jerks. First response (upper record) occurred at position A in figure 3; responses elicited to every succeeding movement until position B was reached (lower record shows last response). Responses were strongest midway between (middle record). Signal of movement as in figure 2. Time in ⅕ second.

that occupied by a single receptor cell. The receptor elements are small, even compared to the exploring spot used in these experiments; consequently, if illumination of but one rod or cone gave rise to the responses in a given optic nerve fiber, charts such as figure 1 would be faithful

representations of the distribution of light associated with the exploring spot. Direct observation of this spot on the retina showed that it was small and sharply focussed, with a halo of scattered light at most only $\frac{1}{100}$ as intense as the spot itself. Yet this spot, at intensities only 4 to 10 times the minimum thresholds for the various fibers, elicited responses over regions many times its own diameter. The observed distributions of sensitivity, with broad maxima several tenths of a millimeter in diameter, in no way resembled the minute exploring spot, only 50 μ in diameter, as they would have if only a single receptor cell had been responsible for the excitation of each optic nerve fiber. Likewise, high sensitivity to slight movement of the spot was not found to be restricted to regions as small as the stimulus spot itself. Finally, the use of the narrow band of shadow upon the uniformly illuminated retina definitely rules out possible effects of scattered light. The sensitivity to slight movements of this shadow, over a region many times its width, offers conclusive proof that many receptor cells are concerned in the excitation of a single optic nerve fiber.

A retinal ganglion cell, therefore, can receive excitatory influences over many convergent pathways; its axon is the final common path for nervous activity originating in many sensory elements. This, of course, is in keeping with the known anatomical organization of the vertebrate retina. It furnishes the functional basis for the spatial effects in the vertebrate retina, observed in experiments on the whole optic nerve by Adrian and Matthews (1927, 1928). They found that the latency of the optic discharge was shorter the greater the area of the retina illuminated, and attributed this to summation of the excitatory effects due to activity in convergent retinal pathways. It is worthy of note that this spatial summation was limited to retinal distances of approximately 1 mm., which is the order of magnitude of the diameter of the receptive fields of the single optic nerve fibers. Moreover, the spatial effects were smaller the greater the retinal distances, in keeping with the diminished effectiveness of the outlying regions of the receptive fields. This diminished effectiveness may be ascribed to a smaller number of receptor elements in a unit area that are in connection with a given retinal ganglion cell, or to a less effective transfer of nervous activity over the longer and less direct pathways from the margins of the receptive field.

The receptive fields of different fibers may overlap considerably (Hartline, loc. cit.). Consequently, illumination of a single point on the retina can produce activity in many different fibers, and illumination of two discrete points may produce activity in many fibers in common. It is for this reason that fine detail cannot be resolved by the peripheral retina. From the standpoint of visual function, it is necessary to consider the distribution of activity among the different fibers of the optic nerve,

elicited by illumination of a particular small element of area on the retina.

A bundle containing a number of active optic nerve fibers may be used to sample this distribution. If not too many fibers are present, it is possible to distinguish the activity in the different ones by means of the loud speaker and the cathode ray oscilloscope. When the responses in such a bundle are tested it is at once apparent that many fibers are excited by a small spot of light (50 μ in diameter), even at intensities close to threshold. Certain of the fibers respond vigorously to the light; these are the ones whose receptive fields are centered close to the stimulus spot. Others give only feeble responses; these either have higher thresholds, or are fibers whose receptive fields are centered at some distance from the stimulus spot, which consequently falls in the less sensitive peripheries of their fields. When the spot of light is tested in a slightly different location on the retina, it is strikingly evident that the composition of the response is changed. Fibers which had been active cease responding, new fibers come into play, fibers which had given strong responses give weak ones, and some of those which had only given slight discharges dominate the response.

It is evident that illumination of a given element of area on the retina results in a specific pattern of activity in a specific group of optic nerve fibers. The particular fibers involved, and the distribution of activity among them, are characteristic of the location on the retina of the particular element of area illuminated. Corresponding to different points on the retina are different patterns of nerve activity; even two closely adjacent points do not produce quite the same distribution of activity, although they may excite many fibers in common. The more widely two illuminated spots are separated the fewer fibers will be involved in common, but it is reasonable to suppose that it is only necessary to have two recognizable maxima of activity in order to resolve the separate spots. It is this spatial specificity of groups of optic nerve fibers, and of the distribution of activity among them, that furnishes the basis for distinguishing the form of the retinal image.

SUMMARY

The region of the retina which must receive illumination in order to elicit a discharge of impulses in a particular optic nerve fiber is termed the receptive field of that fiber. Characteristics of the receptive fields of individual optic nerve fibers from the peripheral retinas of cold-blooded vertebrates (frog, alligator) have been investigated by recording the action potentials in single fibers in response to illuminating various parts of the retina. In several experiments the distribution of sensitivity over the receptive field of a particular fiber has been determined by systematic

exploration of the retina with a small spot of light, noting the responses elicited in the fiber at various locations, and charting the boundaries of the region over which the spot is effective, at various intensities.

The sensitivity to light, referred to a particular optic nerve fiber, is maximal over the central portion of the fiber's receptive field, where the threshold is lower than in the outlying areas, and where intensities above threshold give rise to the strongest responses. The sensitivity is less the greater the distance from this central region, and is usually inappreciable outside an area about one millimeter in diameter. Contour maps of the distribution of sensitivity are given for two examples.

Single optic nerve fibers (of the types responding to "on" and "off," and to "off" only) respond to sudden, slight movements of an illuminated spot, or of a band of shadow on the uniformly illuminated retina, if the moving spot or shadow falls within the receptive field of the fiber. Movements of only a few micra of a small spot or a narrow shadow can elicit responses in a particular optic nerve fiber over a retinal region several tenths of a millimeter in diameter—many times the width of the spot or shadow.

These experiments prove that the receptive field of an optic nerve fiber from the peripheral retina covers an area much greater than that occupied by a single rod or cone. A retinal ganglion cell, therefore, can receive excitatory influences over many convergent pathways; its axon is the final common path for nervous activity originating in many sensory elements. This finding furnishes the functional basis for the spatial effects observed in the peripheral vertebrate retina.

Action potentials recorded from small bundles containing many active optic nerve fibers show that a single small spot of light excites many fibers: the receptive fields of different fibers overlap considerably. The particular fibers activated, and the distribution of activity among them, is characteristic of the location on the retina of the particular element of area illuminated. This spatial specificity of groups of optic nerve fibers, and of their patterns of activity, furnishes the basis for distinguishing the form of the retinal image.

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THE EFFECTS OF SPATIAL SUMMATION IN THE RETINA ON THE EXCITATION OF THE FIBERS OF THE OPTIC NERVE[1]

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In a previous paper (Hartline, 1940) it was shown that a ganglion cell in the peripheral retina of the vertebrate eye is excited by activity in many convergent pathways, from sensory elements distributed over a receptive field covering approximately a square millimeter of retinal area. Illumination of any portion of the receptive field of a retinal ganglion cell will accordingly produce a discharge of impulses in its axon, the strength of the response to illumination of a fixed retinal area usually being greater the higher the intensity of the stimulating light. The present paper will show that the discharge of impulses in a single optic nerve fiber also depends upon the size of the illuminated area. The excitation of a ganglion cell is therefore controlled by the number of active pathways which converge upon it, as well as by the degree of activity in the individual pathways.

Spatial summation in the vertebrate retina has previously been demonstrated by Adrian and Matthews (1927–1928). They showed that the latency of the discharge of impulses in the whole optic nerve of the eel was shorter the larger the area of the retina illuminated, and the latency of the response to four spots of light was shorter than the shortest latency obtained with any of the spots singly. This summation was enhanced by the application of strychnine, indicating that it depended upon the nervous interconnections within the retina. The study of the activity in single optic nerve fibers has now furnished more direct evidence for the convergence of excitatory effects within the retina; the present paper is concerned with the extension of this study to an analysis of spatial summation, in terms of the activity of the individual units of the retina.

METHOD. The method for studying the activity of single optic nerve fibers in the retinas of cold-blooded vertebrates, and for determining the location and extent of their receptive fields has been described in previous papers (Hartline, 1938, 1940). In the present experiments the eyes from

large frogs (R. catesbiana) were used. None of the receptive fields of the fibers studied lay within or near the *area acuta* of the retina; the properties here reported are those of the peripheral retina.

The apparatus for illuminating the retina has likewise been described previously. It provided a beam of light which could be directed upon any part of the exposed retina, more than large enough to cover the region under investigation. The illumination was restricted to any desired area within this beam by means of diaphragms, with apertures of suitable size and shape imaged on the retina. Sharpness of focus was assured, in every experiment, by direct observation of the patterns of light on the retina by means of a dissecting microscope (×32). The diaphragms were readily interchangeable, and slipped into place against mechanical stops in a holder. The accuracy and reproducibility of their alignment in the beam was checked by exposing photographic plates in the place of the retina. Fine adjustments on the diaphragm holder enabled it to be shifted slightly, within the limits of the beam, so that the patterns of illumination could be accurately centered upon the receptive field of the fiber under observation.

RESULTS. In figure 1 are shown oscillograms of the amplified action potentials in a single optic nerve fiber, obtained in response to illumination of the retina with patches of light of various sizes. The areas illuminated, which were circular in shape, had been carefully centered upon the most sensitive portion of the fiber's receptive field, and fell well within its limits. The larger the area of the stimulus patch, the shorter was the latency of response, and, for moderate degrees of stimulation, the higher was the frequency and the greater the number of impulses in the discharge. The fiber used in this experiment was one responding with a burst of impulses at the onset of illumination, and again when the light was turned off (no discharge during steady illumination). Fibers giving other types of response (cf. Hartline, 1938) show a similar dependence of the discharge upon the area illuminated.

Varying the area of the retina illuminated by a fixed intensity thus affects the response in a single optic nerve fiber: this effect, moreover, is exactly similar to that obtained by varying the intensity of illumination upon a fixed retinal area (cf. Hartline, 1938). To permit a comparison, two series of records are shown in figure 1, obtained at two different intensities of illumination. The responses in the right hand column were obtained with an intensity ten times that used in the left. It is to be noted that responses at the higher intensity are comparable to those obtained with areas approximately ten times larger, at the lower intensity. Only the total luminous flux falling upon the retina (area × intensity) is of significance in determining the response of the ganglion cell. This rule has been found to hold, except for very strong stimulation, to within the

limits of accuracy of this method.   It applies only to illumination falling
well within the receptive field of the fiber under observation.

A simple demonstration of this reciprocal relation between the area
and intensity necessary to produce a constant effect in an optic nerve
fiber is furnished by the determination of the threshold intensity, $I_{thresh.}$,
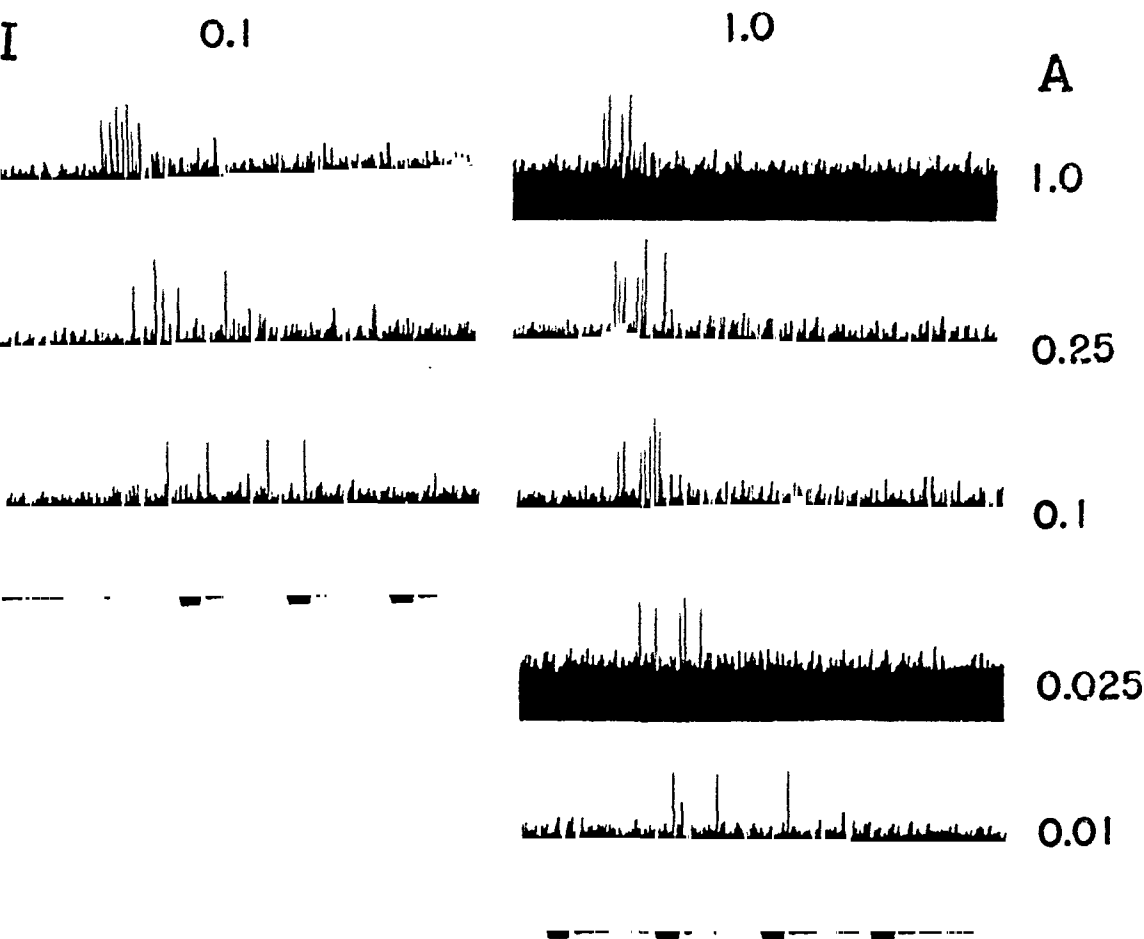
Fig. 1. Oscillograms of action potentials in a single optic nerve fiber from a frog's
retina, showing effect of size of stimulus patch upon the discharge of impulses.
Retina illuminated with circular patches of light, centered on receptive field of the
fiber; relative areas (A) given on right (A = 1 corresponds to 0.006 mm.²).   For the
responses in the left hand column the intensity of illumination was ₁⁄₁₀ that used
for the right hand column.   (I = 1 equivalent to 3.10⁵ meter candles).   Fiber was
one responding with bursts of impulses at "on" and at "off" with no impulses dis-
charged during steady illumination.   Only "on" burst shown here.   Signal of illu-
mination blackens white line above time marker (only shown in bottom records).
Time in ⅕ second.

for various areas, A, of retinal illumination, plotted in figure 2.   The line
through the experimental points has a slope of −1, representing, on this
double logarithmic plot, the relation

$$A \cdot I_{thresh.} = \text{constant.}$$

This relation was demonstrated by Adrian and Matthews (1927–1928) in the optic discharge of the eel's eye; the present experiments show it to be a property of the individual retinal ganglion cells. Its limitation to retinal distances less than 1 mm., as reported by Adrian and Matthews, is due to the fact that the diameter of the receptive field of a ganglion cell is, on the average, of this order of magnitude.

Measurements of the reciprocal of the latent period and of the initial frequency of the discharge of impulses (in the same fiber whose responses are shown in fig. 1) are plotted, in figure 3, as functions of the area of illumination, for various levels of intensity. For moderate degrees of stimulation, these measures of the response increase steadily and approximately linearly with the logarithm of the area illuminated. Curves ob-
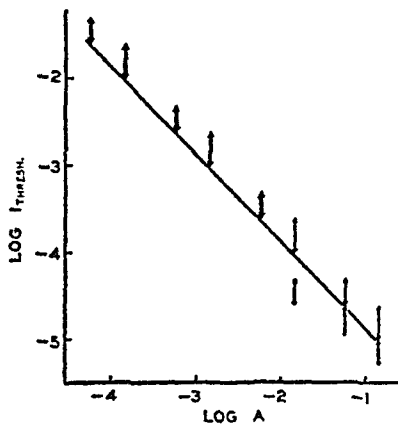
Fig. 2. Relation between area of retina illuminated (A) and threshold intensity, $I_{thresh.}$ for stimulation of a single optic nerve fiber. For each arrow, upper point gives lowest intensity which elicited one or two impulses; lower point gives highest intensity which failed to elicit any response (determinations made to nearest 0.3 or 0.4 log unit). Where duplicate determinations coincided, arrows are drawn heavier. Line drawn through points has slope of −1. (Log I = 0 equivalent to 3.10^5 meter candles; area in mm.^{2}.)

tained at different levels of intensity are separated, parallel to the axis of abscissae, by amounts roughly equal to the logarithms of the ratios of their intensities, in accordance with the reciprocity relation discussed above.

Figure 3 shows that the responses increase with increasing area only up to a certain point. Beyond this point the responses actually decrease with increasing size of stimulus area, although these areas are well within the limits of the receptive field of the fiber. It is furthermore to be noted that the higher the intensity the smaller is the area at which this decrease begins. This effect may also be seen in the right hand column of figure 1, where the response to the largest area contains fewer impulses than the response to the area one-fourth as large. A similar depressing effect on the response has been reported, when the intensity of retinal illumination

on a fixed retinal area is increased above an optimal value (Hartline, 1938). It is as though the ganglion cell can be "overloaded," and the fact that this can be accomplished by increasing the area of the retina illuminated, as well as by increasing the intensity of the light, serves to emphasize the principle that the final response of the ganglion cell is determined by the sum total of activity reaching it over many convergent pathways.

It has been pointed out previously that in these experiments the sensitivity to light of any point on the retina must be defined with respect to the particular optic nerve fiber under observation. The sensitivity, thus defined, is not uniform over the receptive field of a fiber; the outlying portions are less effective in producing responses than is the central region (Hartline, 1940). It is reasonable to suppose that the outlying portions of the receptive field also contribute less to the total summed excitation

a   b
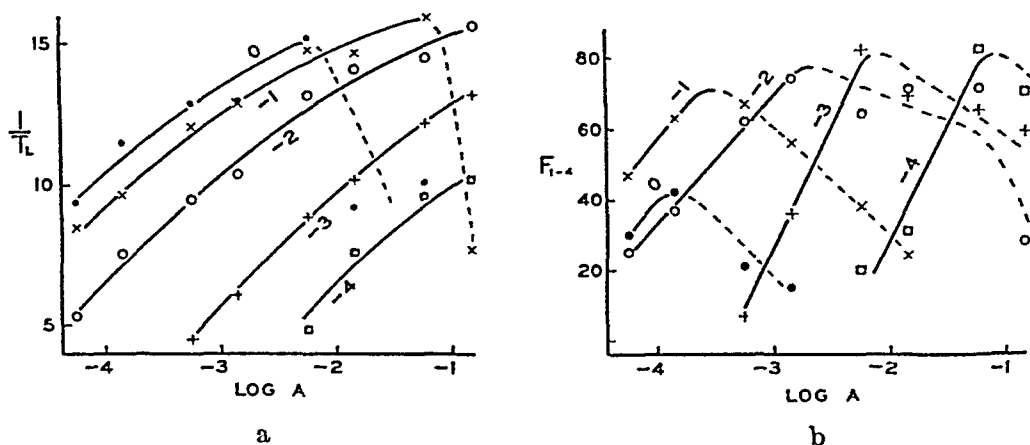
Fig. 3. Relation between area of retina illuminated (A) and response in a single optic nerve fiber, at five levels of intensity. (Measurements of the complete experiment from which the records shown in fig. 1 were selected.) a. Reciprocal of latent period in seconds, T_L, of "on" burst vs. log A (in mm.²). The number on each curve gives the logarithm of the intensity of illumination for that curve (log I = 0 equivalent to 3.10⁵ meter candles). b. Initial frequency of discharge of "on" burst (F_{1-4}; 1st 4 impulses) vs. log A. Numbers on curves give respective values of log I.

of the ganglion cell. To test this point, and to study the relative contributions from the component portions of an illuminated area under different conditions, the following series of experiments have been performed.

A square area, large enough to cover nearly all of the receptive field of a fiber under observation, was subdivided into 25 small squares by means of diaphragms with appropriate apertures. Each of these small areas could be illuminated separately and the response to it compared with the response to illumination of the entire area, or of areas comprising several of the small subdivisions.

The requirements for threshold excitation of a fiber responding at "on" and "off" (only the "on" response recorded) are given in figure 4. The minimal intensity necessary to produce a response was determined for each

small square illuminated alone, and also for areas covered by 4, 9 and 25 of these small squares, as indicated in the figure.   The reciprocals of these threshold intensities are entered in the respective squares, so that the greater the number in a particular square the more effective was that
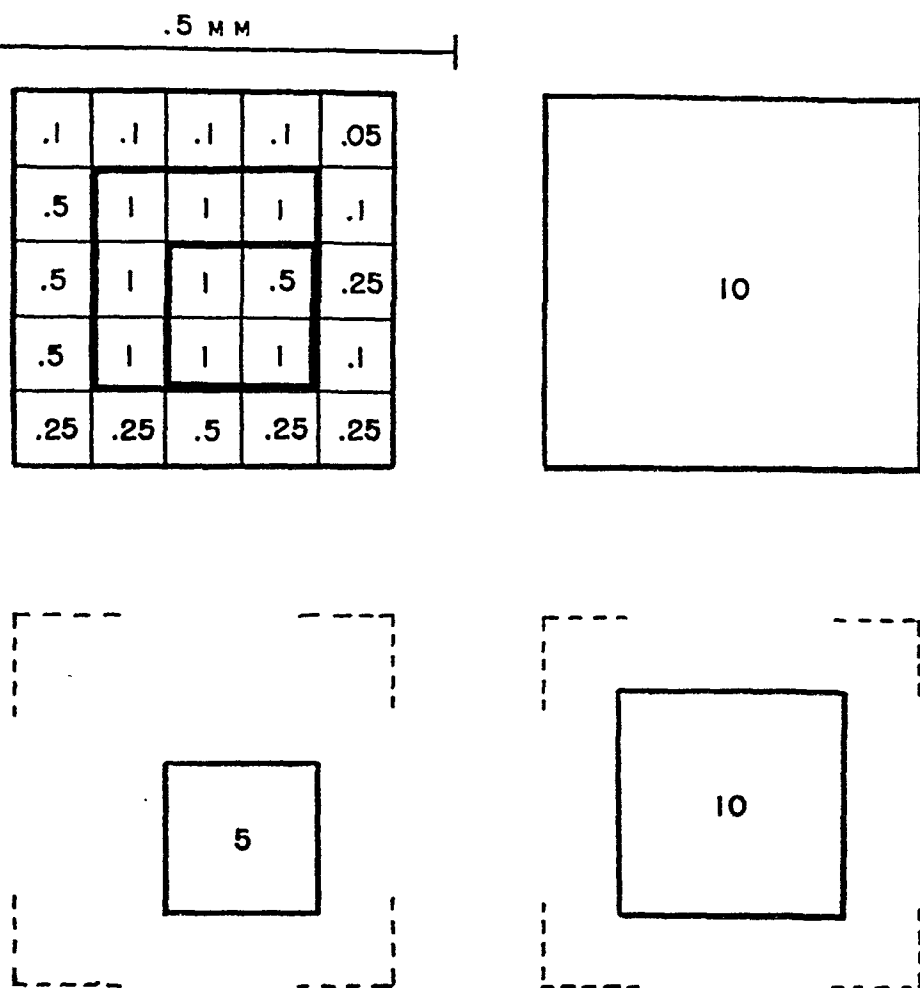
Fig. 4. Chart of the relative effectivness, in stimulating a single optic nerve fiber, of different portions of the fiber's receptive field. "Effectiveness" of a region of the retina defined as reciprocal of threshold intensity for that region. Upper left: numerical values of effectiveness of 25 subdivisions of large square area tested individually. (Comparative scale of retinal distance given above.) Threshold intensity of the most effective subdivisions set equal to 1 (equivalent to 8.10^{-3} meter candles) Lower left: effectiveness of area covering 4 of the central subdivisions (heaviest outline in upper left). Lower right: effectiveness of area covering the 9 central subdivisions (heavy outline in upper left). Upper right: effectiveness of entire large square. · Fiber gave "on" and "off" bursts. "Threshold" taken as the lowest intensity (within 0.3 or 0.4 log unit) which would reliably produce an "on" burst of one or two impulses.

area in producing.excitation of the ganglion cell.   It is to be seen that the region of maximum sensitivity of the receptive field of this fiber was covered by eight of the nine central squares; the 16 border subdivisions

were all considerably less effective. When the larger area covered by
four of the central squares was illuminated, the threshold intensity was
one-fifth that of any of its subdivisions alone; when the still larger area
covered by the nine central squares was exposed the threshold was still
lower—only one-tenth of the threshold of the most sensitive subdivision.
Thus, for the central portion of the receptive field, large illuminated areas
were more effective in exciting the ganglion cell than any of their sub-
divisions. However, when the entire area covered by the 25 small squares
was illuminated, the threshold intensity was not measurably lower than
the threshold of the central region covered by only nine squares. Adding
the 16 border subdivisions did not appreciably increase the effectiveness of
the illumination, in this experiment. To judge from other experiments,
the outlying portions of the receptive field do contribute somewhat to
the total effect, and this might have been observed in the present experi-
ment, had the thresholds been determined more closely. Nevertheless,
the inclusion of less sensitive regions of the receptive field contributes
correspondingly little to the summed effect; illumination of areas entirely
outside the receptive field contributes nothing at all to the excitation of
the ganglion cell.

Spatial summation in the vertebrate retina is thus limited to the re-
ceptive field of the retinal ganglion cell, and its effects are most readily
observable in the more sensitive central portion of that field. A series
of experiments has been performed, designed to analyze the contributions
from component subdivisions of an illuminated area, which in every case
lay well within the receptive field of the fiber under observation.

The experiment of figure 4, just cited, furnishes evidence of the sum-
mation of subliminal excitation. Thus illumination of any single square
at an intensity $1/I = 10$ failed to produce a response, yet this illumination
must have produced some degree of activity in the pathways converging
upon the ganglion cell, for when the nine central squares were illuminated
together, at this intensity, impulses were discharged in the optic nerve
fiber. Another example is furnished by an experiment on a fiber respond-
ing only to the cessation of illumination. At a suitable intensity, illumi-
nation of any one of four small squares singly produced no responses, but
when all four were illuminated together "off" responses were regularly
elicited, consisting of at least 7 impulses, at frequencies of 45 to 60 per
second. Evidently, weak light can produce effects in the individual
subdivisions of an area which are subliminal when they act alone, but
which sum to reach the threshold of the ganglion cell when all act together.
Since the activity in the retinal pathways presumably involves nerve im-
pulses, we must conclude that more than one impulse must reach the
retinal ganglion cell in order to excite a response in its axon.

The experiment of figure 1 shows that spatial summation not only affects

the threshold intensity to which a ganglion cell will respond, but also determines the magnitude of response at intensities above threshold. By testing different subdivisions of an area separately it can be shown, first, that the responses to illumination of a given area may be augmented by subliminal excitation from adjacent regions of the receptive field, and second, that illumination strong enough to elicit responses from each single
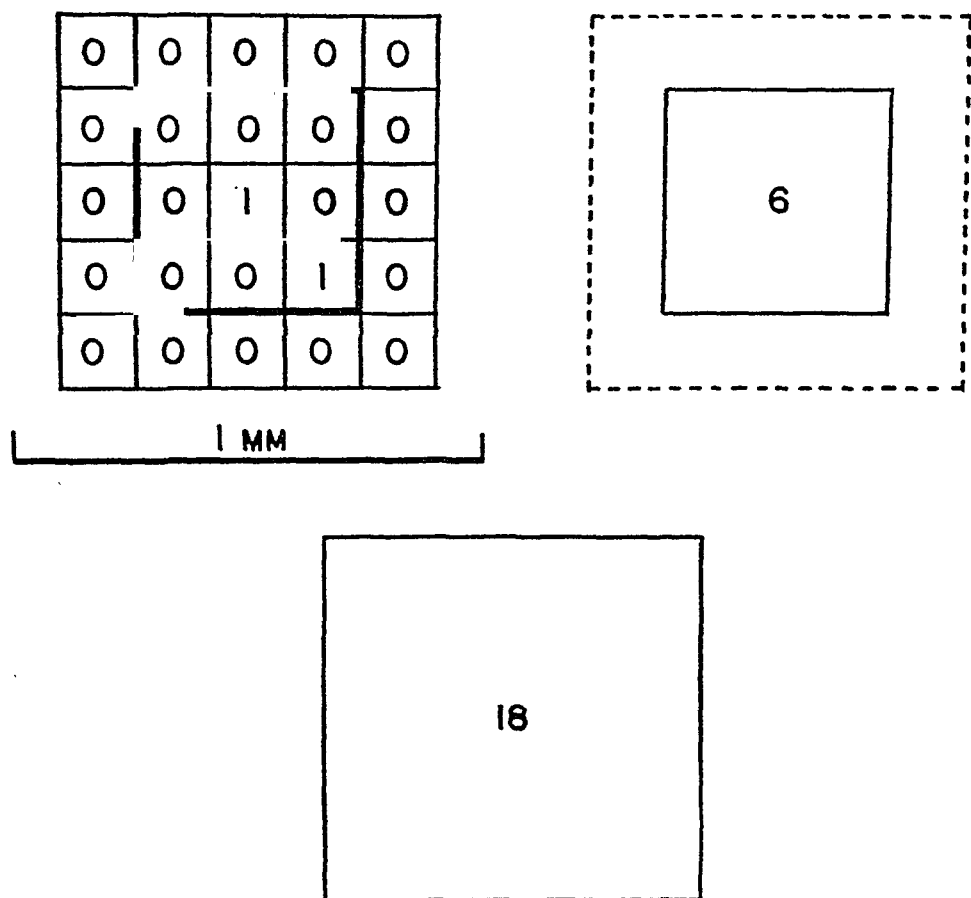
Fig. 5. Chart of the responses of a single optic nerve fiber to illumination of different portions of its receptive field, at a fixed intensity (2.10^{-3} meter candles). Upper left: number of impulses in response to each of 25 subdivisions of large square, tested individually (comparative scale given below). Upper right: response to illumination of area covered by 9 central subdivisions (heavy outline in upper left). Below: response to illumination of entire square. Fiber responded only to "off." Duration of exposure for each test *ca.* 5 sec.

subdivision of an area produces still greater excitation when the total area is exposed.

In an experiment (fig. 5) on a fiber responding only to cessation of illumination, only two of the central squares, out of the 25, would elicit a response (one impulse) when illuminated singly. However, when the area covered by the nine central squares was exposed, at this same intensity,

responses of 6 to 10 impulses, at average frequencies of 10 to 20 per second, were elicited. And when the 16 border subdivisions were added, the response increased to 18 impulses, at 53 per second, although none of these border squares alone could produce any response at this intensity. While
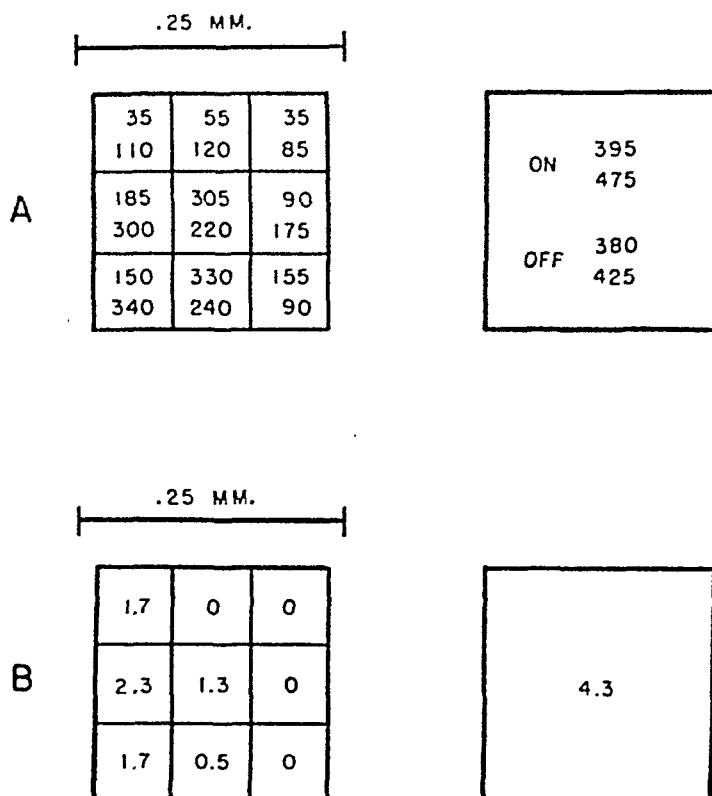
Fig. 6. a. Chart of the responses of a single optic nerve fiber (responding at "on" and at "off") to illumination of different portions of its receptive field, at a fixed intensity (0.3 meter candles). Left: frequencies of discharge (1st 6 impulses) of "on" and "off" bursts (upper and lower numbers, respectively) for each of 9 small squares tested individually (scale of distance given above). Right: frequencies of discharge of the "on" and "off" bursts (upper and lower pairs of numbers, respectively) in response to illumination of entire area covered by the 9 small squares. Upper member of each pair of numbers gives value obtained before testing the small squares, lower member the value afterwards. b. Chart of the frequencies of maintained discharge (13th to 15th second of continuous illumination) of single optic nerve fiber, in response to illumination of each of 9 small squares (left) compared with response to illumination of entire area covered by these squares (right). Scale given above. Intensity 300 meter candles.

it has been shown that border subdivisions contribute less to the summed effect of the illumination than do the more central ones, this experiment shows that their contribution nevertheless may be quite appreciable. This is especially true at low levels of excitation, where a slight increase in the stimulus usually causes a considerable increase in the response.

At an intensity moderately above threshold, the response to illumination of a large area is greater than the greatest response to illumination of any subdivision of this area at this same intensity. Illumination of nine small squares individually at an intensity above threshold resulted in the responses tabulated in figure 6a. When the entire area covered by these nine squares was exposed, at this same intensity, the frequency of the discharge was greater than in the responses of even the most effective subdivision illuminated alone. With fibers of this kind, responding to a change in illumination, both the "on" and the "off" bursts show the effects of spatial summation. A similar result, with a fiber whose discharge was maintained during steady illumination, is shown in figures 6b and 7. The frequency of the steady discharge resulting from illumination of each

Fig. 7. Records of the maintained discharge of impulses in a single optic nerve fiber, showing effects of spatial summation. Top: response to illumination of most effective one of 9 subdivisions of an area of the retina (small square labelled 2.3 in fig. 6, b). Bottom: response to illumination of entire area covered by the 9 subdivisions (labelled 4.3 in fig. 6, b). Records include the 13th to 15th seconds of steady illumination. Intensity 300 meter candles. Time marked in ⅕ second.

of the subdivisions singly is given in the respective square in figure 6b. When the entire area was illuminated, the frequency of the resulting discharge exceeded the highest frequency obtained from any of the small squares alone. Figure 7 shows the records of the responses to illuminating the entire area and to illuminating its most effective subdivision at the same intensity.

As noted previously, excitation above an optimal limit results in diminished responses in an optic nerve fiber. Thus it can happen that the response to the total area is actually less than that to any of its component subdivisions. The fiber, cited above, whose "off" responses illustrated the summation of subliminal effects from four subdivisions of an area, gave the following responses when tested at an intensity 100 times higher. The individual squares, illuminated singly, gave "off" bursts having

initial frequencies of 265, 230, 205 and 195 impulses per second. In response to illuminating the whole area covered by these four squares, at the same intensity, the initial frequency of impulses in the burst was only 175. That this diminished response was due to the excessively high total excitation was shown by reducing the intensity of the light to $\frac{1}{4}$ its previous value; illumination of the whole area then gave a response whose initial frequency was 240 impulses per second. Summation of excitation due to activity in convergent pathways takes place over the entire range of the response of the retinal ganglion cell.

Spatial summation can take place, of course, only where there is convergence of the effects of stimulation. In the more simple eye of *Limulus*, there is no convergence, and the response in a given optic nerve fiber depends only upon the illumination of the sensory cell giving rise to that fiber. Illumination of adjacent areas of the eye has no effect upon this response (Graham, 1932). But where there is convergence there need not be summation; the response in the final common path might be determined solely by the most strongly excited component. This is not so in the vertebrate retina, as was originally evident from the studies of Adrian and Matthews. The present experimental study furnishes direct evidence that the excitation of a single retinal ganglion cell is determined by the summated effects of activity in the pathways converging upon it.

SUMMARY

A study has been made of the action potentials of single optic nerve fibers of the frog's retina, in response to illuminating areas of the retina of various sizes. In these experiments the fibers used were from the peripheral retina, where many receptor elements are connected with each retinal ganglion cell.

The discharge of impulses in a single optic nerve fiber is stronger the larger the area of the retina illuminated, within the limits of the fiber's receptive field. Except for very strong illumination, the responses have a shorter latency and a higher frequency the greater the number of receptors illuminated. The threshold intensity is also lower the larger the area of the stimulating patch of light.

Varying the area of the retina illuminated by a fixed intensity affects the discharge of impulses in a single optic nerve fiber in the same way as varying the intensity of illumination of a fixed area. For threshold excitation and for levels of response above threshold, only the total quantity of light (A·I) determines the response, provided the illumination is confined to the central portion of the fiber's receptive field.

Excitation of a retinal ganglion cell above an optimal limit results in diminished responses in its optic nerve fiber: this effect can be produced by increasing either the intensity or the area of the retinal illumination.

The discharge of impulses in response to illumination of a given area within the receptive field of an optic nerve fiber has been compared with the responses to illumination of subdivisions of this same area. 1. Illumination of the less effective subdivisions in the margins of the receptive field contributes correspondingly little to the summed effect upon the ganglion cell. Illumination of areas entirely outside the receptive field has no effect upon the discharge of impulses. 2. Subliminal effects from the subdivisions of an area can sum to reach the threshold of the ganglion cell when all the subdivisions are illuminated together. From this it is concluded that more than one nerve impulse must reach the retinal ganglion cell, over the pathways converging upon it, in order to excite a discharge in its optic nerve fiber. 3. The discharge of impulses in response to illumination of a given area is stronger than the strongest response from any subdivision of this area, illuminated at the same intensity. This is true provided the ganglion cell is not stimulated too strongly; at very high levels of excitation the response to illumination of the entire area is diminished.

An optic nerve fiber is the final common path for nervous activity originating in many receptor elements of the retina; excitation due to the activity in the retinal pathways converging upon a single ganglion cell summates to determine the response in its optic nerve fiber.

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TEMPERATURE REGULATION IN CHRONIC CERVICAL CATS[1]

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A number of investigators have studied the effects of section of the spinal cord at the cervical level on temperature regulation and have reported conflicting results. The greatest damage to temperature regulation following such an operation was reported by Freund and Strasmann (1912). They found that cervical rabbits, i.e., rabbits with the spinal cord severed in the cervical region, when subjected to very slight changes in environmental temperature showed such marked changes in rectal temperature that the animals could be considered poikilothermic. None of their animals survived more than 11 days and the nutritive state was poor. Sherrington (1924) did not find such great damage to temperature regulation in cervical dogs, although he does state that it was necessary to keep them in a room heated to about 80°F. in order for them to maintain normal body temperatures. His animals were able to pant but they were unable to prevent their temperatures from rising under conditions where normals showed little or no change in rectal temperature. In these dogs he noted an absence of peripheral vasomotor changes and of erection of hair and in addition he noted that shivering only occurred in those regions whose innervation came from above the level of the cord section. Although the majority of the tests which he performed were quite drastic they clearly demonstrated the inadequacy of temperature regulation in cervical dogs as compared to normals. If one examines closely the work of Issekutz et al. (1937) it will be seen that only when his cervical cats were kept in rooms at about 80° were their body temperatures normal.[2] In contrast to the above, other workers have stated that cervical animals possess quite efficient temperature regulation. Popoff (1934) studied dogs in which the spinal cord was destroyed below C_5C_6 and in which the vagi had been sectioned at the level of the thyroid cartilage. He stated that such animals had the same temperature as normal dogs when kept at room temperatures of 50°, and that he had observed vaso-

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[1] Aided by a grant from the Rockefeller Foundation.

[2] In a series of 474 observations of morning rectal temperature on 32 unselected cats at varying room temperatures of about 65 to 85° the mean was 101.37° with a standard deviation of 0.79° (Clark, unpublished observations).

constriction in response to cold and vasodilatation in response to heat. Quite unexplainably he also stated that "das Fell der Hunder bedeckte sich manchmal mit 'Schweisstropfen.' " Except for the latter, somewhat similar results have been obtained by Hermann, Morin and Crier (1938), Hermann, Morin and Galy (1938) and Morin (1938). Thauer (1935) states that after an initial period of thermolability (6-8 days) cervical rabbits can be kept at room temperatures of 65 to 70° and later can withstand room temperatures of 40° without the body temperature falling. He further reported that such animals could withstand overheating as well as normals and that changes in the size of ear vessels occurred in response to changes in environmental temperature. The longest survival which he reported was 70 days.

Because of this difference in results it seemed desirable to reinvestigate the ability of cervical animals to regulate their body temperature. Cats were utilized because considerable information has been accumulated on temperature regulation in normal cats (Clark, Magoun and Ranson, 1939). It was decided to prepare two series of animals in order to determine 1, the amount of recovery in ability to withstand chilling, and 2, the effect of long continued exposure to temperatures, sufficiently low to produce slightly subnormal temperatures, on the ability to withstand chilling. In each cat a laminectomy was performed under ether anesthesia, the cord cut in the lower cervical region and a small segment (1–2 mm.) removed. At autopsy the level of the transection was determined. In the second series the portion of the cord containing the scar was removed, fixed in formalin, embedded in nitrocellulose and stained by the Van Gieson method.

It was found that the care of high spinal animals was more time consuming than difficult. Catheterization was usually necessary for the first 3 days or so, but after that the urine could be pressed out. Occasionally enemas were necessary. The animals were supported on tightly stretched fish net and frequently turned to prevent the development of pressure sores. By careful cleaning perineal ulcers were avoided. The diet consisted of 50 grams of ground beef hearts night and morning. At very infrequent intervals liver or "Pard" was substituted. Most of the cats gained in weight and at autopsy had a thick layer of subcutaneous fat.

First series. At first it was necessary to keep the animals in an incubator set at 90°, but after about a week they were placed in a room with an even temperature of approximately 82°. At weekly intervals throughout the 2½ months that this group was allowed to survive, their ability to react against cold was tested by placing them for from 1 to 5 hours in a room at about 68°. In most of the experiments made to determine the ability of cervical cats to withstand chilling a room designed for paraffin cutting was utilized. This room was connected with a large ice-box by

two openings whose size could be varied by movable slides. A small fan was placed in the upper opening and the fan was connected in series with a resistance box. Proper selection of size of the openings and the amount of resistance in series with the fan made it possible to maintain quite even temperatures in this room. As a matter of convenience the cats were fed their usual ration in the morning and the tests started 5 hours later. This procedure was followed in both series of animals. One month after the operation they were given hot box tests similar to those described by Teague and Ranson (1936).

The ability of the first group of animals to regulate their body temperature in a warm room improved with the lapse of time and was similar in all three. The record of cat 3 may be given as an example. An incubator temperature of 90° was necessary in order to maintain the rectal temperature of this cat within normal limits for the first 3 postoperative days. On the 4th PO (postoperative) day it was possible to lower the incubator temperature to 87°. On this same day this cat was taken out into the room at 79° for $3\frac{1}{2}$ hours and the rectal temperature dropped to 97.1° in spite of constant shivering in those areas with intact nerve supply. On the 6th PO day exposure to a room temperature 79° for $6\frac{1}{2}$ hours did not result in a fall in rectal temperature and on the 8th day 9 hours at 81° did not result in a drop. On the following day the animal was permanently removed from the incubator. Thereafter it was able to maintain normal body temperature in this room, the temperature of which averaged 82° and seldom changed more than 3° in any one day, the maximum variation being from 78° to 87° in the course of the experiment. As long as the temperature was about 85° there was occasional shivering in those areas whose nerve supply came from above the level of the cord section and as the room temperature dropped the shivering became more marked.

The temperatures at which panting began in hot box tests, similar to those described by Teague and Ranson (1936), were found in these 3 cats to be only slightly above the average for normal cats and well within the normal range of variation. Sweating was never observed.

The tests of ability to withstand cold are summarized in table 1. Since normal cats have no difficulty in maintaining the normal warmth of the body at temperatures of 35 to 40° (and probably at much lower temperatures) a failure to regulate at 65 to 70° represents marked impairment. The first test on cats 1 and 3 were made on the 16th and the last on the 65th PO days and the first test on cat 2 was made on the 26th and the last on the 75th PO day. Again the responses of cat 3 may be taken as typical for the group. In the second test, on the 22nd PO day, this cat's rectal temperature dropped from 101.1° to 98.8° after one hour in a room at 68°. By the 56th PO day there had been considerable recovery and the cat's temperature dropped only 2° (from 100.9° to 98.9°) as a result

of 5 hours' exposure to 68°.   There was no further recovery in the next 9 days.   It should be emphasized that this cat's weight continued to increase throughout this period when there was no further improvement in ability to regulate against cold.

Cats 1 and 2 did not show quite as marked recovery as did cat 3 for in the final tests it will be seen that the temperature of cat 2 dropped 5° to 97.4°, that of cat 1 dropped 4.5° to 96.8°, while that of cat 3 dropped only 2.6° to 98.4°.   Usually the greater portion of the drop occurred in the first hour of the test so that changes in the last two hours were slight. Although skin temperatures were not measured the foot-pads and ears felt warm to the touch.   The cats shivered violently in those regions with

TABLE 1

Cold box tests on cats with transections of the spinal cord in the lower cervical region
Rectal and room temperature in degrees Fahrenheit

DATE	CAT 1 RECTAL TEMPERATURE		CAT 2 RECTAL TEMPERATURE		CAT 3 RECTAL TEMPERATURE		COLD ROOM TEMPERATURE	ANIMAL ROOM TEMPERATURE	DURATION OF TEST
	Initial	Final	Initial	Final	Initial	Final			
5/23	102.5	98.8	101.9	97.4	100.8	96.6	65	83	1 hour
5/29	101.8	97.8	101.9	97.8	101.1	98.8	68	82	1 hour
6/5	101.7	97.4	101.3	96.9	100.1	97.9	68	83	2 hours
6/12	101.4	96.2	102.1	96.2	100.9	95.0	65	80	4 hours except cat 3, 2 hours
6/19	99.6	94.2	101.1	96.1	100.6	97.6	65	79	5 hours
6/25	101.9	96.7	101.2	98.0	101.0	99.0	70	81	5 hours
7/2	101.0	97.0	101.8	97.7	100.9	98.9	68	80	5 hours
7/9	100.9	95.5	101.9	97.0	100.9	98.6	68	83	5 hours
7/11	101.3	96.8	102.4	97.4	101.0	98.4	68	86	5 hours

intact nerve supply but shivering never occurred elsewhere.   Erection of hair was never observed.

Second series.   The cats of the second series were placed in a large incubator (set at about 90°).   There they remained for 51 days when 3 were removed and placed in a large refrigerator which had been designed so that acclimatization of spinal cats to cold could be attempted.   This box had been divided into two compartments by a vertical, insulated partition. The two compartments, each of which had its own door, were connected with openings at the top and bottom whose size could be varied at will. The cooling unit was in one compartment and 3 cats were placed on a support of stretched fish net in the other.   The cool air as it entered the cat compartment was mixed with air from the top of the compartment and forced to the bottom by a small electric fan.   Since the temperature

of the room in which the refrigerator stood was usually about 80 to 85°
the compartment in which the cats were kept could be roughly regulated
at any desired temperature from 77° to 50°. The first 3 animals were
kept in the refrigerator for 1½ months. Then they were returned to the
incubator and similar acclimatization was attempted in 2 other animals.
After this the first 3 were returned to the refrigerator, etc. During the
period that an animal was in the refrigerator it was found necessary to
increase the diet 15 grams, giving 65 grams of ground beef hearts night
and morning in order to prevent a loss in weight. At varying intervals
tests were made of the ability of these animals to withstand cold. The
majority of these were made by placing the animals in a room at 65° for
3 hours but in some cases lower temperatures and longer times were used.
A few experiments were performed to determine if sweating occurred in
response to heat and if shivering could be observed in regions whose in-
nervation came from below the level of the cord transection.

In discussing the second series of animals cat 8 will be considered in
detail. For the first 43 PO days it was kept in an incubator which for
the first 3 days was set for 90° and then reduced to 85°. From day to
day there was considerable variation in the incubator temperature which
ranged from 87° to as low as 79°. During this period the cat's rectal
temperature was usually within normal limits but on the one occasion
when the incubator temperature dropped to 79° the morning temperature
was 99.2°. On the 14th PO day the rectal temperature was 99.9° and the
incubator temperature was 84°. After feeding the cat it was placed in
the general animal room where the cage temperature was 74°. In 5 hours
the rectal temperature had fallen to 94.6°. On the 44th PO day the ani-
mal was taken to the refrigerator, the temperature of which was slowly
reduced from 77° to about 72°. The cat ran markedly subnormal tem-
peratures, developed a diarrhea and was returned to the incubator on the
64th PO day. Nine days later it was again placed in the refrigerator
which was now set at about 73°. For the first 3 days the cat would be
left in the cold until its temperature had dropped to below normal levels
and then it would be taken out into the room where the temperature was
about 80–85°. When the cat's temperature had risen to normal it would
be replaced in the refrigerator. After this period the animal remained
in the refrigerator and its rectal temperature was usually about 99°. On
the 81st PO day the cat's temperature dropped 3.2° to 96.3° as a result
of 3 hours' exposure to 65°. The following day it was returned to the
incubator where it remained for 32 days. The morning when it was placed
in the incubator (average temperature 86°) its rectal temperature was 98.0°
and that afternoon it had risen to 104.1° and it did not fall as low as 102°
for 4 days (fig. 1). Thereafter the rectal temperature remained within
normal limits. On the 102nd PO day 3 hours' exposure to 65° resulted

in a drop in rectal temperature of 3.6° to 96.8°. This final temperature was 0.5° higher than after the attempted acclimatization. On the 104th PO day the animal was returned to the refrigerator, the temperature of which was lowered from room temperature to 68 to 70° in 24 hours. The refrigerator temperature remained at this level for 18 days. During
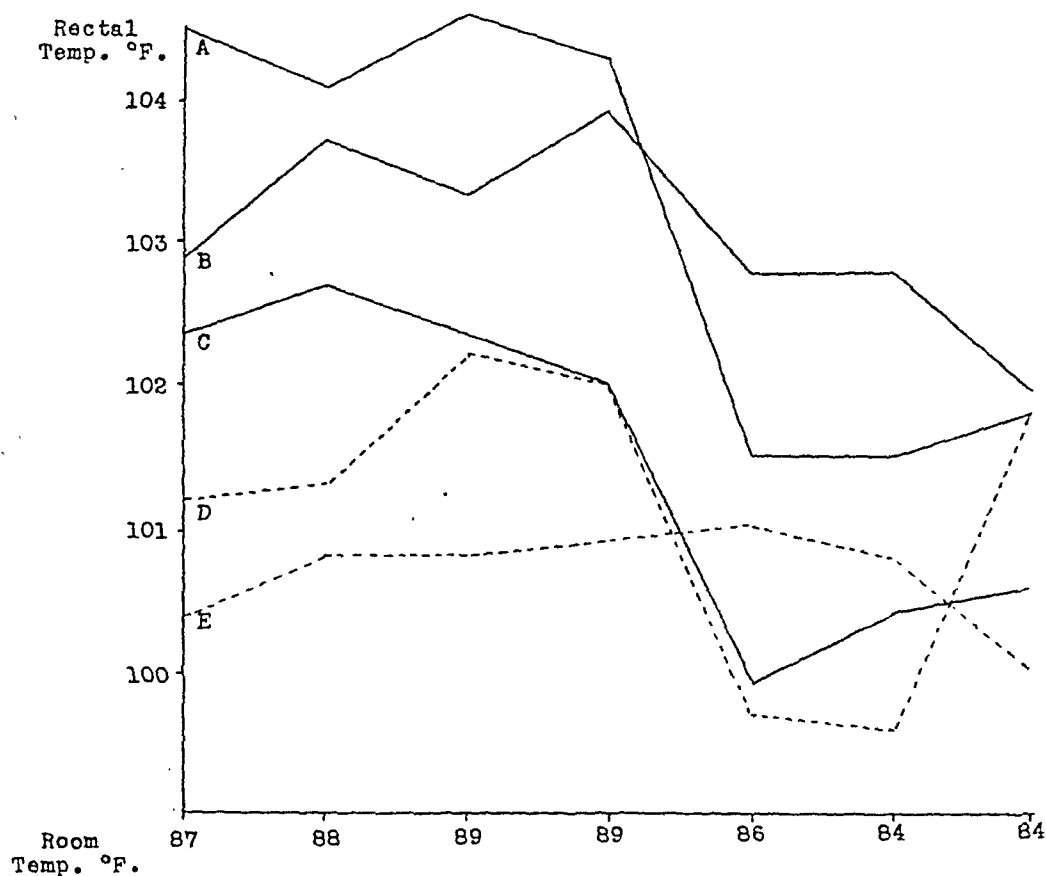
Fig. 1. Shows the effect of returning cats to the incubator after a period of acclimatization to cold. A, B and C represent the temperature records of 3 such cats (nos. 5, 7 and 8) based on daily observations made in the mornings. D represents the average morning temperatures of 3 cervical cats already acclimated to the incubator and E the average temperatures of 2 normal cats under the same conditions. Observe that for the first 5 days the temperatures of the cats acclimated to cold were consistently higher than that of the cats already accustomed to the heat. The wide changes in temperature of the cervical cats are in marked contrast to the almost constant temperature of the normal animals in the same room.

this period the cat's rectal temperature was usually about 96 to 97°. Then the refrigerator temperature was increased to 72 to 74° and the animal's temperature increased to 98 to 99°. This second attempt at acclimatization was successful for on the 137th PO day, after 33 days in the cold, 3-hours' exposure to 65° produced a drop of 2.4° to only 99.6°. After this the animal was kept in the general animal room (75–77°) where it

was able to maintain its rectal temperature within normal limits. At autopsy the section was found to be slightly caudad of the roots of C_7 and microscopic study of the scar revealed that there could be no question of the completeness of the transection.

In table 2 are summarized the results obtained from the second series of cats. Two tests are listed on each animal, i.e., the best test after acclimatization to cold and the best test after acclimatization to heat. By the best test is meant the test in which the final level of the rectal temperature was the highest. In all cases, except as noted in the table, the tests were for 3 hours at 65°. It will be seen that, while only one of

TABLE 2

Evidence of acclimatization in cats with transection of the spinal cord in the lower cervical region

Rectal temperature in degrees Fahrenheit

CAT NUMBER	BEST TEST AFTER ACCLIMATIZATION TO COLD		DAYS IN COLD	POST-OPERATIVE DAYS	BEST TEST AFTER ACCLIMATIZATION TO HEAT		DAYS IN HEAT	POST-OPERATIVE DAYS
	Initial temperature	Final temperature			Initial temperature	Final temperature		
4	102.3	100.0	21	110	101.8	95.9	87	87
5	101.9	99.3	35	147	102.6	98.8	10	100
6	103.0	100.6* 99.2†	11	168	101.9	99.4	10	122
7	101.2	100.1	46	155	102.0	96.6	8	94
8	102.0	99.6	33	135	100.4	96.8	21	100
9	100.6	99.6‡	18	149	99.3	97.5	135	135
10	100.0	97.9‡	18	149	99.9	97.2	112	112

* Three hours at 55°F.

† Six hours at 55°F.

‡ In animal room average temperature 75°F. These two animals were never kept in ice box.

the cats was able to maintain its rectal temperature above 99° after 3 hours' exposure to 65° when acclimatized to heat, 6 of the 7 animals were able to do so after acclimatization to cold. The exception, and one of the other cats, were never placed in the refrigerator but had been in the general animal room where the temperature averaged 75°. Cat 6 after acclimatization to cold was not seriously chilled by exposure to 55° for 6 hours.

As mentioned previously these tests of ability to prevent chilling were started 5 hours after feeding. An attempt was made to determine the influence of this short period between feeding and tests. Routine tests at 65° were made on cats 6 and 7 on the 186th and 183rd PO days respec-

tively. The rectal temperature of cat 6 remained within normal limits while that of cat 7 dropped 2.4° to 99.4°. On the following morning food was withheld and a cold test was started 16 hours after the previous evening's feeding. As a result of 3 hours' exposure to 65° the rectal temperature of cat 6 dropped 2.3° to 98.4° and that of cat 7, 5.4° to 97.2°. It is questionable which of the two tests truly represents the ability of these animals to withstand chilling. Bruhn (1940) found it necessary to withhold food for 24 hours in his metabolism studies of midbrain dogs.

Very shortly after these cats recovered from the anesthetic it was possible to elicit a crossed-extensor reflex. Throughout their survival period this reflex could consistently be obtained with only slight stimulation. In most of the animals it was not possible to elicit a scratch reflex until about 3 weeks after the operation, but after that it could be obtained easily. In those animals which survived 3 months or longer mass reflexes were often seen. A pinch of the tail or foot-pad, or a scratch on the side would produce at first a to-and-fro movement of the tail, a crossed extensor reflex or a scratch reflex respectively, then if the stimulus were repeated a few times, the activity would begin to spread, more and more muscle groups would become involved, and finally there would be vigorous twistings of the trunk, urination and occasionally defecation. After the spread had begun it was not necessary to continue the original stimulus. If the bladder or colon were distended it was much easier to secure this response. At first the nictitating membranes were relaxed but after a week or 10 days they returned to the usual position. At times, however, the nictitating membranes again relaxed and would remain relaxed for widely varying periods of time ranging from a few minutes to several days. This phenomenon could not be correlated with differences in the level of the lesion nor with the condition of the animal. The pupillary response to light was normal. Pinching the rear foot-pad produced little, if any, change in pupil size, but on the contrary, a light pinch of the fore pad caused a marked dilatation. This is in agreement with the work of Ury and Gellhorn (1939) who stress the importance of inhibition of the third nerve in reflex dilatation in response to pain. Maes (1939), who transected the spinal cord at C_1 and maintained the cats under artificial respiration on a heating pad for 3 to 6 hours, stated that he was able to elicit treading, raising of the pelvis and tail movements characteristic of "heat" as a result of tapping the perineum when the animals (previously treated with estrogens) were held in a crouching position. While elevation of the pelvis was not observed, the other responses could be obtained consistently upon tapping the perineum of this series of cats. This was done while the animals were lying on their sides and it is possible that if they had been held in a crouching position that elevation of the pelvis, also, would have occurred.

In order to secure greater accuracy in the observations on shivering than can be attained by the sense of touch (Sherrington, 1924) use was made of electrical recording. Electrodes embedded in Cambridge electrode jelly were applied to the skin overlying both extremities of the humerus and femur on one side. The cat's temperature was reduced to 95° by placing the animal in the cold compartment of the refrigerator at about 40°. Then the animal was placed in a shielded room and the electrodes connected with an amplifier and cathode ray oscillograph. In the muscles of the fore limb, whose innervation came from above the lesion the
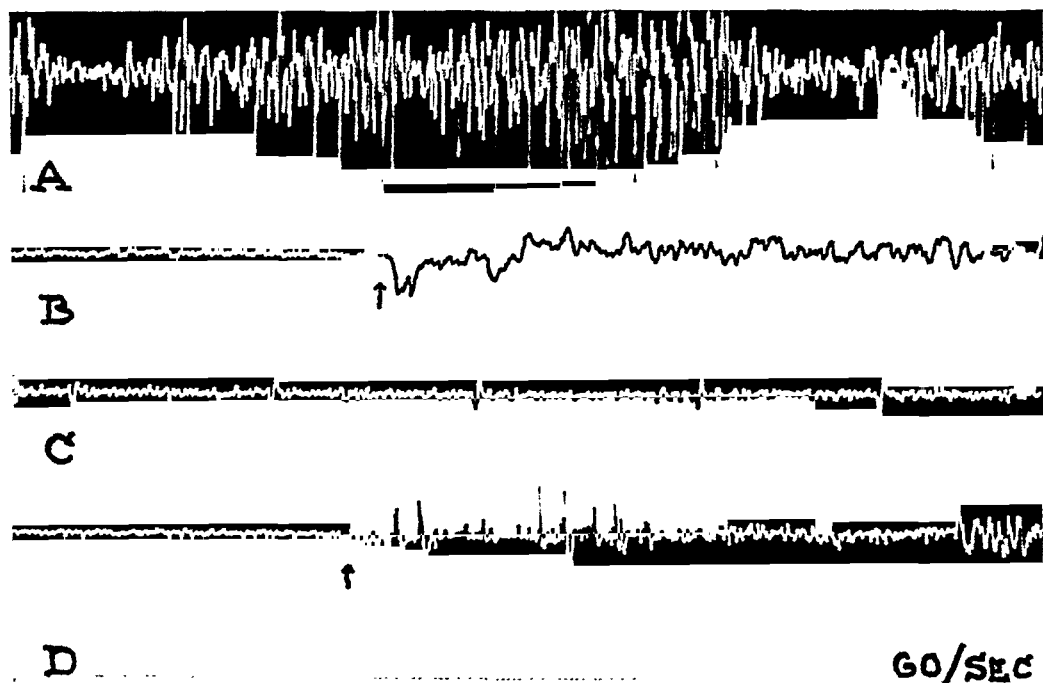
Fig. 2. Muscle potentials from the fore limb A and hind limb B of a cat with low cervical transection of the spinal cord with a rectal temperature of 95° and C from the fore limb and D from the hind limb of the same cat after its temperature had been raised to 101.6°. The arrows indicate pinching of the opposite hind foot and the muscle potentials which follow are associated with the crossed extensor reflex.

shivering was almost continuous (fig. 2A). The record from the hind limb (B) was taken a minute later. At the arrow the foot-pad was pinched and the ensuing activity is that of the crossed extensor reflex. Then a heating pad was placed beneath the cat and its temperature was raised to 101.6° and a record taken from the front leg (C) and from the hind leg (D). The periodic activity of the heart muscle is seen in C and the crossed extensor reflex is seen in D following the arrow which indicates pinching the foot. In the chilled animal the shivering which was so obvious in the record from the fore limb was absent in the hind limb

and it disappeared in the fore limb when the animal's temperature was raised.

Observations on sweating are rendered difficult because of evaporation which can completely mask a slow secretion. To obviate this difficulty fingers from rubber gloves were used to cover the cat's hind feet. Several normal cats were heated till panting occurred and in each case there was definite evidence of sweat inside the glove finger when it was removed. Four of the cervical cats were similarly tested and in no case was there any indication of sweat within the glove finger. Mass reflexes in patients with transected spinal cords are accompanied by sweating (Head and Riddoch, 1917) but in these cats, although mass reflexes accompanied by defecation and urination could be produced, sweating was never observed during these paroxysms.

Since cat 6 showed during the entire postoperative period considerably better reactions to cold than any of the others and was finally able to stand an exposure for 6 hours to a temperature of 55° and emerge from the test with a rectal temperature of 99.2° the question arises whether in this case the cord was completely cut. The cords from the second series of cats were fixed, embedded and cut in serial transverse sections and stained by the Van Gieson method. In none of the cords could evidence be found that the section had been incomplete and no bundle of uncut fibers could be traced across the scar. But in cats 4, 5, 6 and 7 the scar joining the severed parts of the cord together was so thin and irregular that it was equally impossible to be sure that no fibers crossed the scar joining the two ends together. If there were any such fibers they were few in number and could not be identified under the microscope. In the remaining 3 cats such a thick scar was found that there could be no question of the completeness of the cord section.

Since shivering never occurs in muscles whose innervation comes from below the level of the lesion and since erection of hair was never observed, there seem to be only two possible explanations for the acclimatization to cold. There must either be an increase in the basal metabolic rate or a chronic peripheral vasoconstriction. There is abundant evidence that changes in the thyroid gland occur as a result of exposure to cold (Uotila, 1939; Starr, 1940; Baillif, 1937) and that changes in the hypophysis may be seen (Baillif, 1938). This indicates that the rate of secretion of thyroxine may be increased as a result of long-continued exposure to cold. Indeed, Ring (1939) has recently shown that sufficient exposure to cold will increase the resting metabolism of white rats an average of 16 per cent. Since in the cold tests the ears and foot-pads of the cats acclimatized to cold did not seem perceptibly colder than those of cats from the incubator the other possibility—a chronic peripheral vasoconstriction—does not appear likely.

CONCLUSIONS

The results of these experiments show that cats with transection of the lower cervical cord are unable to make the adjustments necessary for maintaining a normal body temperature when there occurs a sudden and considerable fall in environmental temperature. But these animals are still capable of a limited slow adjustment to cold, an acclimatization. This increased ability to withstand cold which is acquired as a result of the gradual lowering of the environmental temperature is lost after the animals have been kept again in a warmer environment and is probably due to an increased metabolic rate.

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THE HEMOLYTIC ACTION OF CHYLE[1]

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It has been previously demonstrated that samples of lymph collected from the lacteals and thoracic ducts of dogs from 3 to 5 hours or more after the ingestion of fat are strongly hemolytic (Johnson and Freeman, 1938), by making ordinary red blood cell counts on mixtures of equal quantities *a*, of whole blood plus lymph[2] (about 0.015 cc. of each), and *b*, of blood plus Ringer's solution. Almost without exception the red blood cell counts made on blood and chyle mixtures were lower than the counts made on blood and Ringer's solution mixtures. The supernatant fluid of centrifuged samples of blood and chyle mixtures also showed definite evidence of hemolysis. These findings have been confirmed repeatedly, using the same technique with slight modifications.

What is this factor in chyle which causes hemolysis? The substance is found in the lacteals and thoracic duct only after fat-feeding. Lymph obtained from these sources in fasting animals or from cervical or foot lymphatics in fat-fed animals does not hemolyze red blood cells when mixed in equal quantities with whole blood. Thus, it is a substance which appears in the lymph only during the transport of products of fat absorption. The following substances were considered:

1. *Cholesterol.* This has been shown repeatedly to be antagonistic to the hemolytic action of such substances as fatty acids and saponin (Meyerstein, 1912; Brinkman, 1929). Therefore, it was not considered to be an important causative factor.

2. *Neutral fat.* This is probably not an important factor since mixtures of equal quantities of blood plus milk or cream give the same red blood cell counts as blood mixed with Ringer's solution.

3. *Bile salts.* Sodium taurocholate is definitely hemolytic and might be involved in the hemolytic action of chyle. Since good quantitative methods for bile salt determination are lacking, no chemical estimations were

[2] Heparin was used as an anti-coagulant for both blood and lymph in some cases. In other instances, intravenous injection of Chlorazol Fast Pink BKS (Modell, 1939) prevented coagulation of both blood and lymph.

attempted. However, the introduction of bile or bile salts into the intestine yielded lymph possessing little or no hemolytic activity. Therefore, it seems that the rôle of bile salts in the hemolytic activity of chyle is probably insignificant.

4. *Osmotic pressure.* No direct measurements of the osmotic pressure of chyle were made in this study, but the work of several investigators (summarized by Drinker and Field, 1933) indicates that the total osmotic pressure of lymph is even slightly higher than that of serum. Furthermore, many mixtures of potently hemolytic chyle and whole blood showed crenation of the red blood cells. These findings suggest that salt osmotic pressure changes are not involved in the hemolytic action.

5. *Hydrogen ion concentration.* Determinations were made of hemolytic potency[3] and the pH of lymph samples collected under oil. The pH was determined by means of a glass electrode and was found to remain almost constant throughout an experiment, having no apparent relationship to hemolytic potency. Table 1 is a typical protocol.

TABLE 1

The hemolytic potency and pH of thoracic duct lymph collected after fat feeding

TIME AFTER FAT FEEDING	HEMOLYTIC POTENCY OF LYMPH	pH OF LYMPH
hours	*per cent*	
$3\frac{2}{3}$	20	7.357
4	34	7.357
$4\frac{1}{2}$	6	7.341
$4\frac{3}{4}$	3	7.349

6. *Temperature.* The temperature of the mixtures was kept constant without varying the results.

7. *Enzymes.* Chyle samples were collected and divided into two portions. One portion was heated to 75°C. for 10 minutes; the other was untreated. No constant or significant differences in hemolytic potency of heated and unheated samples were observed.

8. *Glycerol.* In an earlier report (Johnson and Freeman, 1938) it was suggested that glycerol might be responsible for the hemolytic action of chyle. Other investigators (Ramond and Flandrin, 1904) had demonstrated large amounts of glycerol in the lacteals and thoracic ducts of dogs,

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[3] Throughout the paper, percent hemolysis is used as an expression of hemolytic potency of samples tested. It is determined by the formula $\dfrac{C_R - C_L}{C_R} \times 100$, where C_R is the control red blood cell count made upon mixtures of equal quantities of whole blood plus Ringer's solution and C_L is the red blood cell count made upon mixtures of equal quantities of whole blood plus the sample (usually lymph) whose hemolytic potency is being determined.

but they reported concentrations equally as high in the portal and peripheral blood streams, where presumably no hemolysis occurs. In the present study, glycerol was placed directly into washed, intact intestinal loops, or glycerol was fed with diets not containing fat in a number of dogs. This yielded thoracic duct lymph which was not hemolytic. These considerations seem to indicate that glycerol is probably not the substance which causes hemolysis.

9. *Free fatty acids and soaps.* Of the substances found in the gut during fat digestion, there remain free fatty acids and soaps. Might not quantities of these sufficient to cause hemolysis escape resynthesis into neutral fat and be absorbed into the lacteals? Several lines of evidence supported this hypothesis. Placing oleic acid or sodium oleate in washed intestinal loops of dogs gave intensely hemolytic chyle. Also, earlier workers (Faust and Tallqvist, 1907) fed fatty acid to one dog and a cholesterol ester to another, getting lymph with hemolytic power. They extracted the lymph with fat solvents, made a suspension of the dissolved material and showed that this also was hemolytic.

In an attempt to discover whether free fatty acid and soap were responsible for the hemolytic action of chyle, quantitative analyses for soap and free fatty acid were made. The analytic method employed combined features of methods published by several workers (Boyd, 1936; Bloor, 1915; Fowweather, 1926; Stoddard and Drury, 1929). Briefly, the method was as follows:

A 3:1 alcohol-ether mixture (both freshly redistilled) was acidified with 1 cc. of 3 per cent hydrochloric acid for each 50 cc. One cubic centimeter of the fluid containing fatty acid and soap was run slowly into 35 cc. of the mixture. This was heated to about 60°C. for two minutes, stirred to prevent superheating, cooled and centrifuged. The supernatant fluid was then poured into a 50 cc. volumetric flask and the residue was re-extracted with 15 cc. of the alcohol-ether. Two 20 cc. aliquots of the alcohol-ether extracts were placed in evaporating dishes containing extracted and washed sand and were evaporated to dryness with gentle heat on a steam bath. The dry residues were each dissolved in 25 cc. of petroleum ether and were filtered through fat-free filter paper. The duplicate samples of petroleum ether extract were heated almost to boiling and were titrated with approximately 0.02 normal sodium ethylate to a pink that did not change, using phenolphthalein as the indicator. Blanks, checks and knowns were run through the whole procedure.

Checks on the method consisted in adding known quantities of soap and fatty acid to lymph or Ringer's solution, and then analysing these solutions and emulsions as described above. The results are shown in figure 1, in which known milligrams added are plotted against milligrams detected by analysis. If the recovery were perfect, all the points would

fall on the straight line drawn into the figure. The distribution of the points shows that not quite all of the added soap or acid was recovered. However, the results were considered sufficiently accurate for the purposes of this study.

Next, the extent of hemolysis produced by mixing red blood cells with Ringer's solutions to which known amounts of fatty acid and soap were added in vitro was determined. These findings are recorded graphically in figure 2, in which the milligrams of added soap or fatty acid are plotted against the hemolytic potencies. Lines are drawn connecting determinations made on individual animals. Although the fluctuations from animal to animal are considerable, it is apparent that there is a positive correlation between quantity of fatty acid or soap added and the hemolytic potency of the solution or emulsion.

Finally, samples of thoracic duct lymph were analysed for soap and free fatty acid content. In fasting dogs, whose lymph showed no hemolytic activity, the quantities of soap and free fatty acid ranged from 1.0 to 2.0 mgm. per cubic centimeter—too little to produce detectable hemolysis. Values ranging from 3.3 to 6.3 mgm. per cubic centimeter were found in the course of $3\frac{1}{2}$ to $4\frac{1}{2}$ hours following fat-feeding. In a few instances, soap and free fatty acid were determined separately on each sample. The concentration of free fatty acid alone in chyle rarely reached 1 mgm. per cubic centimeter. Using somewhat different methods, other investigators have also found free fatty acids and soaps in chyle. Faust and Tallqvist (1907) found about 12 mgm. of soap plus free fatty acid in the thoracic duct lymph of a dog fed an oleic acid-cholesterol ester, and even more in a dog fed oleic acid. Munk (1880) also presents larger values for free fatty acid and soap than were found in the present study. Hoppe-Seyler (1879) calculated that from 2.5 to 4.0 mgm. of soap enter the blood stream by way of the chyle every minute following the ingestion of fat. The work of Freeman and Friedemann (1935) indicates that about 12 per cent of the fatty acids in chyle are uncombined. Lastly, Artom and Peretti (1935) found that 2 per cent of the fatty acids were present in chyle as soaps and free fatty acids.

Are the values reported here sufficient to account for the hemolysis observed? In each sample analysed chemically, the hemolytic potency was also determined. In figure 3 the results are plotted, showing the relationship of the quantity of soap or fatty acid detected in lymph to the hemolytic potency of the lymph. The line drawn in figure 3 is derived from the data shown in figure 2 by connecting the averages of the points on figure 2. Points in figure 3 lying above the line represent lymph samples containing at least enough soap or fatty acid to account for the extent of hemolytic potency observed. That the line in figure 3 is not placed too low is borne out by observations of Edwards (1939), McPhedran (1913), and Zinck, Clark and Evans (1922) who were able to produce definite

hemolysis with solutions of fatty acids and soaps even less concentrated than the solutions used in the present study.

The data in figure 3 seem to indicate that, in general, the quantity of soap and fatty acid found in chyle is adequate to account for the hemolytic potency. One can only speculate on the failure of the points to distribute themselves along a straight line. Perhaps the variations in the resistance of corpuscles from different animals to these agents are partly responsible. The data of figure 2 support this view. Perhaps there are variations in the anti-hemolytic action of serum, or in the concentrations of cholesterol, an anti-hemolytic agent. Several workers (Liebermann, 1907; Noguchi, 1907; Meyer, 1908; Meyerstein, 1912; Zinck, Clark, and Evans, 1922;
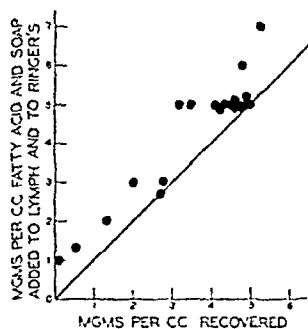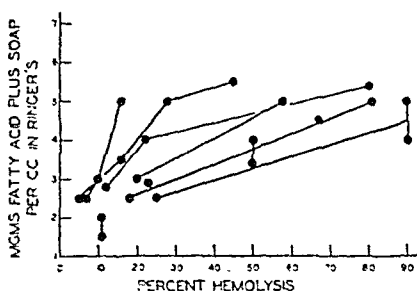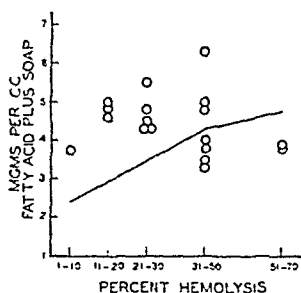
Fig. 1   Fig. 2   Fig. 3

Fig. 1. Relation of added milligrams of soap and free fatty acid to milligrams as detected by analysis. The straight line indicates the theoretical location of the points if the recoveries were complete.

Fig. 2. Relation of amount of soap or fatty acid added to Ringer's solution (in vitro) to extent of hemolysis observed when this was mixed with equal quantities of whole blood. Lines are drawn through determinations made on the cells of individual animals.

Fig. 3. Relation of hemolytic potency to quantity of fatty acid and soap determined by chemical analysis. The line drawn represents the averages of points plotted in figure 2.

Brinkman, 1929) have shown that serum exerts a protective action against hemolysis by soaps and fatty acids. The presence of some anti-hemolytic activity is suggested by the distribution of the points to the left, in figure 3, since these points represent chyle samples containing more than enough fatty acid or soap to account for the hemolytic potency. Finally, there may be variable quantities of insoluble calcium soaps formed (Brinkman and Szent-Gyorgyi, 1923).

Whether or not the figures reported are of significance, physiologically or pathologically, remains to be determined. It has been suggested (Johnson and Freeman, 1938) that absorption of these hemolytic agents into the lymphatics may be of adaptive value, since instead of entering capillaries at once, where red cells might be damaged, the hemolysins enter the blood stream only after they have been diluted by lymph from

many parts of the body. Further, the diluted hemolysins are poured into a stream of blood returning from many regions, instead of initially entering and mixing with the blood of the intestine alone. It remains to be demonstrated that the direct entrance of these substances into the capillaries of the portal circulation would destroy or damage red blood cells.

SUMMARY AND CONCLUSIONS

1. The existence of a hemolytic agent in thoracic duct lymph, during absorption of ingested fat, is amply confirmed. Other lymph is not hemolytic.

2. Evidence is presented that this hemolytic agent is not cholesterol, neutral fat, bile salts, enzymes, glycerol, or changes in osmotic pressure, hydrogen ion concentration, or temperature.

3. The soap plus free fatty acid content of chyle is 3.3 to 6.3 mgm. per cubic centimeter during rapid fat absorption. Most of this is probably soap.

4. These quantities are sufficient to account for the hemolytic action of chyle.

5. The duct lymph of fasting dogs contains too little fatty acid or soap to produce hemolysis.

6. The possible sgnificance of these findings is discussed.

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THE ACTION OF IONS ON THE FROG HEART

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In studying the effect of varying the concentration of Na, K or Ca ion in Ringer's solution on the frog-heart rate, I have found that the heart rate is depressed if the concentration of any one of these ions is sufficiently greater or less than that generally considered normal for this solution. This depression is usually of a progressive nature and appears to be due simply to the abnormality, or toxicity, of these experimental Ringer's solutions. However, within certain limits of concentration, the heart continues to beat for long periods without any great change in rate (Spealman, 1938). These limits may tentatively be considered to be "physio- ' logically normal."

My previous studies have concerned only the heart rate; and it is possible that the "normal" limits of concentration would be different for other activities of the heart. Unfortunately, most investigators who have made fairly extensive studies on the effect of ions on the cold-blooded heart have usually used concentrations which deviate widely from the values ordinarily used in Ringer's solution. (See Daly and Clark, 1921; Andrus and Carter, 1922, for the more recent, extensive studies.) It appears likely that they studied effects produced by rather abnormal solutions.

The present investigation was carried out to establish the approximate "normal" concentration limits of the different ions (Na, K, Ca and H) with respect to certain activities of the frog heart (the amplitude and duration of the ventricular response, ·the systolic and diastolic tone, and the a-v time interval) and to study the effect of varying the concentration of these ions, when kept within "normal" limits, on these activities.

METHODS. In all experiments, the heart was removed from the frog and perfused through the sinus venosus. The apex of the heart was attached to a heart lever. In the experiments on the amplitude of the ventricular response and on the tone (table 1), the extent of the excursions of the lever and the systolic and diastolic lengths of the heart were read from a millimeter scale held near the tip of the heart lever but not touching it; this procedure avoided the variable frictional load present when a lever is in contact with a surface. In the experiments on the a-v time interval and on the duration of the ventricular response (table 2), tracings

TABLE 1

The amplitude of the ventricular response and the tone in Ringer's solutions of various compositions

Table 1a gives a summary of the results obtained with Ringer's solution containing various concentrations of $CaCl_2$. The concentrations of $CaCl_2$ in mols per liter are given at the heads of the columns. The solutions otherwise have the composition of normal Ringer's solution. Tables 1b, 1c and 1d are constructed in the same manner and show the results obtained when the KCl concentration, the NaCl concentration, and the pH, respectively, were varied. Also included in table 1c are data obtained with Ringer's solution containing 0.1 mol per liter of dextrose (second column from right) and with a Ringer's solution containing half the normal concentration of NaCl and 0.1 mol per liter of dextrose (column at extreme right).

The amplitude of the ventricular response is expressed as a decimal fraction of the value obtained in normal Ringer's solution. For each experimental value of the amplitude of the ventricular response, two other figures are given; the upper-right figure represents the change in systolic tone, and the lower-right figure, the change in diastolic tone. ((+) indicates an increase and (−) a decrease in tone.) The numerical values given for the tone changes were obtained by dividing the change in length of the heart by the amplitude of the ventricular response. (See text for a more complete explanation.) In the experiments marked with an asterisk, the heart rate was maintained constant at 40 beats per minute by electrical stimulation; in the other experiments the heart was allowed to beat at its own natural rate. 0.00 indicates the heart stopped contracting before measurements could be made. — indicates that no value was taken.

a. $CaCl_2$

EXP.	0.00025	0.0005	0.001	0.002 (N. RINGER'S)	0.003	0.004	0.008
1	—	−0.33 0.65 +0.02	−0.13 0.87 0.00	0.00 1.00 0.00	+0.09 1.06 +0.03	+0.09 1.12 −0.03	—
2	—	−0.44 0.56 0.00	−0.37 0.63 0.00	0.00 1.00 0.00	—	+0.16 1.16 0.00	—
3	—	−0.19 0.81 0.00	—	0.00 1.00 0.00	—	+0.04 1.04 0.00	—
4	—	−0.25 0.77 −0.02	—	0.00 1.00 0.00	+0.15 1.13 +0.02	—	—
5	—	−0.33 0.67 0.00	—	0.00 1.00 0.00	+0.03 1.03 0.00	+0.12 1.12 0.00	—
6*	−0.57 0.30 +0.13	−0.24 0.76 0.00	—	0.00 1.00 0.00	—	−0.04 0.95 +0.01	−0.04 0.89 +0.07

TABLE 1—*Continued*

a. CaCl₂—*Continued*

EXP.	0.00025	0.0005	0.001	0.002 (N. RINGER'S)	0.003	0.004	0.008
7*	−0.21 0.58 +0.21	−0.14 0.81 +0.05	—	0.00 1.00 0.00	—	−0.07 0.99 −0.06	−0.10 0.79 +0.11
8*	−0.48 0.45 +0.07	—	−0.08 0.92 0.00	0.00 1.00 0.00	—	−0.03 0.95 +0.02	−0.07 0.87 +0.06

b. KCl

EXP.	0.00025	0.0005	0.001	0.002 (N. RINGER'S)	0.004	0.008	0.016
1	—	—	−0.25 0.77 −0.02	0.00 1.00 0.00	−0.12 0.86 +0.02	—	—
2	—	—	−0.05 0.95 0.00	0.00 1.00 0.00	0.00 0.99 +0.01	—	—
3	—	—	+0.02 1.02 0.00	0.00 1.00 0.00	0.00 1.03 −0.03	—	—
4	—	—	−0.18 0.88 −0.06	0.00 1.00 0.00	0.00 1.04 −0.04	—	—
5*	—	−0.06 0.85 +0.09	−0.07 0.92 +0.01	0.00 1.00 0.00	+0.06 1.00 +0.06	−0.23 0.73 +0.04	—
6*	−0.15 0.85 0.00	+0.10 1.08 +0.02	+0.06 1.06 0.00	0.00 1.00 0.00	−0.05 0.98 −0.03	−0.02 1.00 −0.02	−0.06 0.82 +0.12
7*	−0.20 0.59 +0.21	−0.01 0.84 +0.15	+0.03 1.00 +0.03	0.00 1.00 0.00	—	+0.01 1.00 +0.01	−0.45 0.38 +0.17

c. NaCl

EXP.	0.05	0.10 (NORMAL RINGER'S)	0.13	0.20	0.10 + 0.10 M DEXTROSE	0.05 + 0.10 M DEXTROSE
1	—	0.00 1.00 0.00	+0.07 1.06 +0.01	—	—	—

TABLE 1—*Concluded*
c. NaCl—*Continued*

EXP.	0.05	0.10 (NORMAL RINGER'S)	0.13	0.20	0.10 + 0.10 M DEXTROSE	0.05 + 0.10 M DEXTROSE
2	—	0.00 1.00 0.00	+0.21 1.13 +0.08	—	+0.15 1.10 +0.05	—
3	—	0.00 1.00 0.00	+0.11 1.11 0.00	—	+0.05 1.10 −0.05	—
4	—	0.00 1.00 0.00	+0.14 1.14 0.00	—	−0.12 0.90 −0.02	—
5*	−0.34 0.52 +0.14	0.00 1.00 0.00	−0.04 0.99 −0.03	−0.11 0.69 +0.20	−0.02 0.98 +0.00	−0.06 0.94 0.00
6*	−0.21 0.68 +0.11	0.00 1.00 0.00	+0.04 1.02 +0.02	−0.14 0.75 +0.11	−0.03 0.96 +0.01	−0.13 0.84 +0.03
7*	−0.18 0.49 +0.33	0.00 1.00 0.00	+0.01 1.03 −0.02	−0.15 0.69 +0.16	+0.02 1.01 +0.01	+0.06 1.04 +0.02

d. pH

EXP.	3.0	4.3	5.8	7.0	7.7 (N. RINGER'S)	8.3	9.6	10.6
1*	0.00	—	—	−0.01 0.95 +0.04	0.00 1.00 0.00	—	—	—
2*	—	—	—	—	0.00 1.00 0.00	−0.04 0.98 −0.02	—	0.00
3*	—	−0.62 0.26 +0.12	—	−0.03 1.00 −0.03	0.00 1.00 0.00	+0.03 1.02 +0.01	—	−0.18 0.64 +0.18
4*	—	—	−0.13 0.85 +0.02	+0.02 1.02 0.00	0.00 1.00 0.00	—	−0.13 0.88 −0.01	−0.17 0.65 +0.18
5*	—	—	−0.12 0.86 +0.02	−0.01 0.98 +0.01	0.00 1.00 0.00	—	−0.13 0.87 0.00	—

TABLE 2

The a-v time interval (A-V) and the duration of the ventricular response (DVR) in Ringer's solutions of various compositions

The column at the extreme left shows the concentration of the substances studied in mols per liter; otherwise each solution has the composition of normal Ringer's solution. All values obtained in these experiments are expressed as decimal fractions of the values obtained in normal Ringer's solution. Average values are given in the column at the extreme right. The dash (—) indicates that no value was taken with that solution in the experiment.

	EXPERIMENT 1	EXPERIMENT 2	EXPERIMENT 3	EXPERIMENT 4	EXPERIMENT 5	AVERAGE
a. CaCl$_2$						
0.0005						
A-V...................	0.94	0.93	0.82	—	1.06	0.94
DVR.................	0.85	0.81	0.84	—	0.72	0.81
0.001						
A-V...................	—	1.00	0.83	1.11	0.98	0.98
DVR.................	—	0.84	0.86	0.91	0.85	0.87
0.002*						
A-V...................	1.00	1.00	1.00	1.00	1.00	1.00
DVR.................	1.00	1.00	1.00	1.00	1.00	1.00
b. KCl						
0.001						
A-V...................	1.61	1.21	1.77	1.20	1.37	1.43
DVR.................	1.33	1.03	1.10	1.20	1.22	1.18
0.002*						
A-V...................	1.00	1.00	1.00	1.00	1.00	1.00
DVR.................	1.00	1.00	1.00	1.00	1.00	1.00
0.004						
A-V...................	0.90	1.03	0.78	0.69	0.71	0.82
DVR.................	0.86	0.74	0.76	0.94	0.64	0.79
c. NaCl						
0.100*						
A-V...................	1.00	1.00	1.00	1.00	1.00	1.00
DVR.................	1.00	1.00	1.00	1.00	1.00	1.00
0.125						
A-V...................	1.02	0.91	0.95	0.94	1.00	0.96
DVR.................	1.00	0.97	1.25	1.00	1.00	1.04
0.100†						
A-V...................	1.25	1.02	0.91	—	1.07	1.06
DVR.................	1.21	1.37	1.38	—	1.28	1.31

TABLE 2—*Concluded*

	EXPERIMENT 1	EXPERIMENT 2	EXPERIMENT 3	EXPERIMENT 4	EXPERIMENT 5	AVERAGE
d. pH						
6.9						
A-V...................	1.03	1.01	1.03	—	1.00	1.02
DVR.................	1.00	0.98	0.91	—	0.97	0.97
7.7*						
A-V...................	1.00	1.00	1.00	1.00	1.00	1.00
DVR.................	1.00	1.00	1.00	1.00	1.00	1.00
8.4						
A-V...................	0.94	0.94	—	1.00	1.00	0.97
DVR...............	1.00	1.12	—	0.90	1.03	1.01

* Normal Ringer's.
† + 0.10 M/L dextrose.

were made on a constant speed kymograph. In these experiments a lever was also attached to one of the atria. The a-v time interval was determined by measuring the distance on the drum between the upstroke of the two levers; the duration of the ventricular response was determined by measuring the distance between the point at which the lever started to rise and a more or less arbitrary point at which the lever returned to the base line.

The procedure in all experiments was to make the necessary measurements or tracings, first, with the heart perfused with normal solution, then with the heart perfused with the experimental solution, and finally with the heart again perfused with normal solution. In the experiments on the amplitude of the ventricular response and on the tone, 3 measurements were made at 5 minute intervals with each solution; in the experiments on the a-v time interval and on the duration of the ventricular response, 3 measurements were made at 10 minute intervals with each solution.

In most of the experiments, the heart was allowed to beat at its normal rhythm. In some of the experiments (marked with asterisks in the tables) the heart was driven by means of electrical stimulation at a rate of 40 beats per minute; in these experiments, the sinus venosus was removed from action by tying the cannulating ligature on the atrial side of the white crescentic line. The reason for using artificial stimulation in some of these experiments was to rule out the possibility that a change in the heart rate was responsible for some of the effects found; for it is known that the heart rate affects certain other activities of the heart, e.g., the amplitude of the ventricular response.

Each value reported in the tables for the amplitude of the ventricular

response, for the duration of the ventricular response, and for the a-v time interval was calculated by dividing the average of the values obtained with the experimental solution by the average of the values obtained with normal Ringer's before and after perfusion with the experimental solution.

The values given for the tone changes require some comment. The readings actually taken were the positions of the tip of the heart lever on the millimeter scale during complete systole and complete diastole. An elevation of the position of the lever, in either systole or diastole, occurring during perfusion with an experimental solution is termed plus (+) in the table; a depression is termed minus (−). These correspond respectively to an increase in tone (decrease in length of the heart) and to a decrease in tone (an increase in length of the heart). In order to show quantitatively the changes in systolic and diastolic lengths, the actual differences in millimeters between the systolic positions and between the diastolic positions of the lever during perfusion with normal Ringer's solution and during perfusion with the experimental solution are divided by the amplitude of the ventricular response (extent of excursion of the lever) occurring during perfusion with normal Ringer's solution. The figures so obtained show the quantitative changes in the systolic and diastolic lengths of the heart which result in a change in the amplitude of the ventricular response. For example, in table 1a, the figures under the column headed 0.0005 M/L Ca ion in experiment 1 signify that the amplitude of the ventricular response occurring with that solution was 0.65 the amplitude of the ventricular response occurring with normal Ringer's solution, i.e., there was a decrease in the amplitude of contraction of 0.35 of the original amplitude. Of this decrease, 0.33 was due to an increase in systolic length of the heart and 0.02 was due to a decrease in diastolic length of the heart.

RESULTS. Table 1 shows the effect on the amplitude of the ventricular response and on the systolic and diastolic tone (length) of varying the Na, K, and Ca ion concentrations, the pH, and the osmotic pressure. The table shows that the amplitude of the ventricular response becomes smaller if the concentration of any of these ions deviates too far from normal. With concentrations closer to normal, the heart is not greatly affected except by Ca ion which causes an increase in the amplitude of the ventricular response and an increase in systolic tone as the concentration of this ion is increased.

Table 2 shows the effect on the a-v time interval and on the duration of the ventricular response produced by variation of the Na, K, and Ca ion concentrations, the pH, and the osmotic pressure. The various ions were studied only in concentrations close to normal. This was done because the heart will not maintain a constant beat outside this range, and it is undesirable to use artificial stimulation. The table shows that K ion causes a decrease in the a-v time interval and a decrease in the duration of the ventricular response as the concentration of this ion is increased.

Ca ion causes an increase in the duration of the ventricular response as the concentration of this ion is increased. Addition of dextrose (increasing the osmotic pressure) caused an increase in the duration of the ventricular response. The heart is not significantly affected in these respects by the other ions.

Discussion. When the concentration of any one of the positive ions in Ringer's solution is sufficiently different from that usually used in normal Ringer's solution, the amplitude of the ventricular response decreases. This decrease appears to be associated with an increase in systolic length and a decrease in diastolic length of the heart; the result is that there is less difference between the contracted and the relaxed lengths of the heart. Because these changes are not related to any particular ion, it is probable that this behavior is simply a non-specific response of the heart to abnormal or toxic solutions. A further fact supporting this conclusion is that this behavior of the heart is usually progressive; that is, when the heart is perfused with one of the solutions which cause this behavior, the systolic and the diastolic lengths of the heart become more nearly the same as time goes on. Finally, as I have previously mentioned, in studying the effects of ions on the heart rate, I have found that outside of certain limits of concentration, the rate usually became progressively less (Spealman, 1938). These limits are practically the same as the concentration limits (which may be inferred from table 1) outside of which the contractile ability of the heart shows evidences of failing. I think it is justifiable to conclude that the concentration limits suggested in table 1 of this publication (also see Spealman[1], 1938) can be considered as the approximate "normal" limits of concentration for each of these ions for the perfused frog-hearts used here (Rana pipiens, winter frogs).

There have been but few studies carried out within these "normal" limits. However, the effect of Ca ion on the amplitude of the ventricular response has been studied over the "normal" range by Clark (1928), and it is well known that increasing the concentration of this ion increases the amplitude of the ventricular response as table 1 shows. In fact McLean and Hastings (1934) have used this as a method for determining ionized calcium in mammalian blood. This increase is due entirely to a decrease in the systolic length of the heart.

It is usually stated that changes in K ion concentrations also affect (in the opposite direction to Ca) the amplitude of the ventricular response. In fact, Trendelenburg (1921) gives curves showing this. It is likely that the decrease in amplitude of the ventricular response found by Trendelenburg was obtained only by K ion concentration sufficiently high to be

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[1] In this publication, the values for Na ion concentration are too high by 0.016 M/L. This was due to the fact that the $NaHCO_3$ concentration was taken to be 0.0175 M/L; the concentration actually was 0.00175 M/L.

"abnormal." As mentioned above, a decrease in the amplitude of the ventricular response can be obtained easily by sufficiently increasing or decreasing the concentration of any of the ions of Ringer's solution. I found no change in the amplitude of the ventricular response when K ion concentration was varied in the region of its normal value.

Ca ion seems to be the only factor studied which, in reasonable concentration limits, affects the amplitude of the ventricular response or the tone. The studies with Na ion deserve some comment, for an additional factor, the osmotic pressure of the solution, becomes important here. I have varied the Na ion concentration 1, by changing the NaCl concentration (which also allows the osmotic pressure to vary), and 2, by decreasing the NaCl concentration and adding dextrose in sufficient concentration to maintain the osmotic pressure (see column to extreme right of table 1c). In neither case was the amplitude of the ventricular response or the tone greatly changed within "normal" limits. (The first 4 experiments with 0.13 M/L NaCl show some increase in the amplitude of the ventricular response; however, this effect was not obtained in the 3 experiments in which artificial stimulation was used. The moderate effect in the first 4 experiments is probably not significant.) Addition of dextrose to normal Ringer's solution (to increase the osmotic pressure) did not greatly affect these properties of the heart. It might be expected that the amplitude of the ventricular response in the experiments without artificial stimulation would be changed somewhat, since dextrose decreases the heart rate; for it is well known that decreasing the heart rate may affect the amplitude of contraction. In 2 of these 3 experiments there is an increase in the amplitude of the ventricular response; but in the 3 experiments where artificial stimulation was used, there was no effect on the amplitude of contraction. It may be concluded that neither Na ion concentration nor osmotic pressure appreciably affect these properties of the heart.

As table 2 shows, the duration of the ventricular response becomes greater as the K ion concentration is decreased, and, to a smaller extent, as the Ca ion concentration is increased. The Ca ion action may be simply secondary to the effect of Ca ion on the amplitude of the ventricular response; for it is reasonable to expect that if the muscle contracts more completely, it will take a longer time for the response to occur.

Increasing the osmotic pressure with dextrose also increases the duration of the ventricular response to some extent. This is probably due to the fact that the heart rate is decreased by dextrose; for I have found (unpublished results) that the duration of the ventricular response is increased as the heart rate is decreased (artificial stimulation).

K ion appears to be the only factor studied here which appreciably affects the a-v time interval. As my experiments show, the a-v time interval is decreased as the K ion concentration is increased.

CONCLUSIONS

1. All positive ions ordinarily included in Ringer's solution must be present within certain concentration limits in order that the perfused heart can function properly for a long period of time. Outside these limits all ions produce rather similar depressive changes in the heart activities studied. These changes appear to be non-specific, and due to the fact that the solutions are too abnormal to allow the heart to function properly.

2. Certain activities of the heart are specifically modified when the concentrations of certain positive ions are varied within what may be termed their "normal" limits. An increase in Ca ion concentration causes an increase in the amplitude of the ventricular response, an increase in systolic tone, and an increase in the duration of the ventricular response. An increase in K ion concentration causes a decrease in the a-v time interval and a decrease in the duration of the ventricular response. The activities of the heart investigated here are not appreciably affected by variation within "normal" limits of the Na ion concentration, the osmotic pressure, or the pH.

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THE EFFECT OF CLIMATE UPON THE VOLUMES OF BLOOD AND OF TISSUE FLUID IN MAN

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The blood and tissue fluid constitute important links in the chain of mechanisms by which man regulates his body temperature. It is reasonable to expect, therefore, that changes of climate may cause marked variations in the volume of either the blood or tissue fluid and also changes in their composition. Barcroft (1, 2, 3) while on his way to the Peruvian Andes found that the blood volume (calculated from cell volume measured with CO) increased about 35 per cent in three members of this party while they were sailing through the tropics, and decreased again as they got further south to cooler weather. More recently, Bazett (4) has reported increases of 30 to 40 per cent in the blood volume (calculated from the plasma volume measured with vital red) as the summer heat struck Philadelphia, and 8 to 48 per cent in six subjects who, in the wintertime, spent a few days in a room at 32°. In the course of some studies during the past summer in the hot and humid "Delta" region of Mississippi, in the town of Benoit, halfway between Memphis and Vicksburg, we were able to make 65 determinations of plasma volumes and of "available fluid" in three groups of individuals. The first group consisted of ten laboratory workers who were measured both in Boston and Mississippi, the second of white sharecroppers native to Mississippi, and the third of colored sharecroppers native to Mississippi. In addition, we made a few measurements on colored boys and on less active adults.

The techniques used were essentially those of Gregersen and Stewart (5) and of Gibson and Evelyn (6). However, as we made some slight modifications, our method will be described briefly. The subject came to the laboratory in the morning fasting and lay down for half an hour. Four cubic centimeters of blood were then drawn from the antecubital vein, and 1 gram of NaSCN dissolved in 20 cc. of water was injected intravenously, followed immediately by 15 mgm. of the dye T1824 dissolved in 10 cc. of water. Four blood samples of 4 cc. each were then drawn at approximately 15-minute intervals, usually from the opposite antecubital vein, followed by two more samples at hourly intervals, the

subject remaining on the bed. The blood was transferred to a hematocrit tube of 4 cc. capacity and containing 1 drop of heparin solution. (This contained 0.8 per cent NaCl and 400 units of heparin (Connaught Laboratories, Toronto) per cc.) The samples were centrifuged for an hour, and the concentration of dye in the plasma determined on the Evelyn photoelectric colorimeter. For the determination of the NaSCN, 1 cc. of the plasma was then squirted rapidly from an accurately calibrated syringe into 10 cc. of 10 per cent trichloroacetic acid and the precipitated proteins were removed by filtering. Eight cubic centimeters of the filtrate were added to 3 cc. of 10 per cent acid ferric nitrate and the resulting color read within a minute or two. It was observed at the high temperatures and under the rather intense light prevailing in the laboratory in Mississippi that the color faded sufficiently rapidly to produce errors if the solutions were allowed to stand over 5 minutes after the color had developed.

The calculations for the plasma volume and the available fluid followed the lines laid down by Gregersen (5) and Gibson (6), but the blood volume was calculated from the plasma volume and the hematocrit without making the rather large correction suggested by Gregersen to allow for the greater proportion of plasma in the capillary blood. The "available fluid" was calculated directly from the concentration of NaSCN in the plasma from the last three samples of blood, i.e., those taken at one hour, two hours, and three hours after the injection. The "interstitial fluid" was calculated by subtracting from the "available fluid" the plasma volume + 70 per cent of the cell volume (the cells being about 70 per cent water). These terms and figures are arbitrary and take no account of the uncertain but probably small amount of NaSCN which enters certain cells, e.g., those of the salivary glands. They are the same terms and figures that Gregersen and Stewart used, with the one important exception noted above, namely, that we made no correction for the increased proportion of plasma in the capillary blood. Our reasons for omitting this correction are that its proper value is not known, that we feel Gregersen's figure is too large, and that as many other authors, especially Barcroft (2) and Gibson (6), have not made this correction our figures will be more readily comparable with theirs if we omit it. The work of Manery and Hastings (7) suggests that it may not be possible to divide tissues into those which do take up NaSCN and those which do not, but rather into those which take more and those which take less. It is probable therefore that our values run high rather than low.

Judging from the consistency of the values obtained from different samples in the same experiment and also from repeated determinations on the same individual, the probable error of the method was about 2 per cent for the plasma volume and between 1.5 per cent and 2 per cent for the available fluid. In many experiments the probable error was less

than this, but there were also many in which the plasma, though clear to the eye, gave readings on the colorimeter suggestive of a slight cloudiness, perhaps attributable to a large meal rich in fat the preceding evening. In these the probable error was 2 per cent to 4 per cent for the plasma volume, but the available fluid determinations were not affected. On the other hand, though repeated determinations of available fluid on one subject under the same conditions gave fairly consistent results, there was a disquieting amount of difference between individuals and big changes with changed conditions. It is possible that these variations may be due in part to changes in the permeability of certain cells to NaSCN as well as to changes in the amount of interstitial fluid.

The climate in this region of Mississippi during the summer is uniform, hot, and damp. In the period between June 6 and August 16, during which our observations were made, the highest temperature was 101°F. and the lowest 68°F. The mean of the daily highs was 93°F. and the mean of the lows 73°F. These were the outdoor temperatures; indoors the fluctuation was undoubtedly less. The mean humidity at 6:30 a.m. was 88 per cent, at noon 59 per cent, and at 6:30 p.m. 65 per cent. The average precipitation was 2.8 inches per month and it came principally as thunder-showers. The "percentage of sunshine" was 65. These figures for temperature and rainfall are taken from the United States Weather Bureau data for Greenville, Mississippi, a town about twenty miles south of Benoit and very similar in climate. The humidity and sunshine figures are averages of the observations made at Vicksburg and Memphis, the nearest cities on each side of Benoit at which such observations are made, and similarly situated with respect to the river.

Our results with respect to blood and plasma volumes agree qualitatively but not quantitatively with Barcroft's and with Bazett's. They are given in the tables but may be summarized as follows. Of the ten Whites who had determinations made upon them in Boston (or elsewhere in the North) during the winter (November through April inclusive), nine lost weight, on the average 2 kgm., and one gained 1 kgm. on going to Benoit. In some individuals part of this loss occurred before reaching Mississippi. In five the hematocrit reading increased, in one it was unchanged, in four it went down, the average increased insignificantly. The plasma volume increased in eight and decreased in two subjects, the greatest increase being 420 cc. and the greatest decrease 245 cc. The average went up 4.2 per cent in contrast with Barcroft's 35 per cent and Bazett's 30 per cent. The average plasma volume per kilogram and per square meter of body surface increased a little more (because of the loss of weight), 6.1 per cent and 4.7 per cent respectively. Since the average hematocrit value was practically unchanged, the percentage change in blood volume was almost the same as in plasma volume. In general, those individuals who showed the largest

increases in plasma volume showed decreases in their hematocrit readings, while those whose plasma volume increased but little or decreased in the hot weather showed increases in their hematocrit readings.

There was no consistent change in plasma volume between the first set of determinations, which were made within ten days of arrival, and the second set made a month later. Some individuals went up, some down.

TABLE 1

Plasma volumes in Boston, Massachusetts, and in Benoit, Mississippi

	AGE	HEIGHT	BODY WEIGHT, KILOGRAMS			PLASMA VOLUME, CC.			PLASMA VOLUME, CC. PER KGM. OF BODY WEIGHT			PLASMA VOLUME, LITERS PER M². OF BODY SURFACE		
			Boston	Mississippi	Δ	Boston	Mississippi	Δ	Boston	Mississippi	Δ	Boston	Mississippi	Δ
A. Ten White laboratory workers														
Highest......	48	188	84.0	79.0	+1.0	3,845	3,880	+420	55.3	52.9	+6.9	2.11	1.98	+0.21
Lowest.......	26	165	61.5	61.2	−5.0	2,970	3,230	−245	40.1	45.6	−3.2	1.67	1.81	−0.13
Average.....	34.3	175	74.6	72.6	−2.0	3,436	3,581	+145	46.4	49.3	+2.8	1.82	1.90	+0.086
			% change		−2.7	% change		+4.2	% change		+6.1	% change		+4.7
B. Seven White sharecroppers in Mississippi														
Highest......	21	186		71.5			3,900			58.5			2.01	
Lowest.......	17	170		53.5			3,110			46.9			1.75	
Average.....	18.9	177.3		64.6			3,410			53.0			1.89	
C. Twenty-one Colored sharecroppers in Mississippi														
Highest......	24	181		72.3			3,900			59.2			2.14	
Lowest.......	17	165		55.3			2,870			44.4			1.59	
Average.....	20.1	174		61.9			3,300			53.6			1.89	
D. Two Colored servants in Mississippi														
Highest....	23	194		77.9			4,450			57.2			2.13	
Lowest.......	22	178		74.1			3,950			53.3			2.06	
Average	22.5	186		76.0			4,200			55.3			2.10	
E. Four Colored boys from sharecroppers' families in Mississippi														
Highest.....	11	154		33.7			2,010			60.0			1.68	
Lowest.......	8	142		32.9			1,900			57.8			1.58	
Average......	9.5	148.3		33.4			1,953			58.8			1.63	

The average plasma vol./kgm. rose slightly due to the loss of weight. The figures in the tables are average values.

In comparing individuals with one another, the volumes per square meter of surface area proved to be more constant than the volumes per height or weight. This agrees with Rowntree and Brown (8). Though there were considerable variations in the plasma vol./m². within a group, the average variation of individuals from the mean of their group being

4.6 per cent, there was a remarkable constancy in the means for the groups. Thus the laboratory Whites, who increased 4.7 per cent, or from 1.82 liters plasma vol./m². in Boston to 1.90 liters in Mississippi, were in Mississippi within 1 per cent of the values for the native Negroes and the native Whites, which were both 1.89 liters. This extremely close correspondence is no doubt fortuitous, but it indicates the improbability

TABLE 2

Blood volumes in Boston, Massachusetts, and in Benoit, Mississippi

	SURFACE AREA		HEMATOCRIT			BLOOD VOLUME, CC.			BLOOD VOLUME, CC. PER KGM. OF BODY WEIGHT			BLOOD VOLUME, LITERS PER M². OF BODY SURFACE		
	Boston	Mississippi	Boston	Mississippi	Δ	Boston	Mississippi	Δ	Boston	Mississippi	Δ	Boston	Mississippi	Δ
A. Ten White laboratory workers														
Highest......	1.99	1.97	47.6	47.2	+2.8	6,540	7,055	+605	91.9	97.3	+13.1	3.50	3.56	+0.40
Lowest.......	1.70	1.70	39.8	41.4	−4.0	5,560	5,950	−200	75.2	81.4	−2.4	2.90	3.20	−0.10
Average......	1.896	1.876	44.0	44.05	+0.05	6,125	6,400	+275	82.8	87.9	+5.1	3.24	3.40	+0.16
			% change	+0.1		% change	+4.5		% change	+6.2		% change	+4.9	
B. Seven White sharecroppers in Mississippi														
Highest......		1.94		47.9			7,290			111.0			3.75	
Lowest.......		1.61		41.6			5,560			85.0			3.19	
Average......		1.801		45.2			6,237			97.1			3.46	
C. Twenty-one Colored sharecroppers in Mississippi														
Highest......		1.89		46.5			6,740			111.0			3.89	
Lowest.......		1.60		39.7			5,130			80.0			2.89	
Average......		1.752		43.0			5,782			93.9			3.31	
D. Two Colored servants in Mississippi														
Highest......		2.09		44.4			8,000			103.0			3.83	
Lowest.......		1.92		41.9			6,800			91.8			3.56	
Average......		2.00		43.2			7,400			97.4			3.70	
E. Four Colored boys from sharecroppers' families in Mississippi														
Highest......		1.23		38.4			3,260			97.4			2.65	
Lowest.......		1.16		35.8			3,034			90.8			2.54	
Average......		1.20		37.4			3,115			93.7			2.60	

that under the conditions of our experiments there were any changes in blood volume comparable to those found by Barcroft or Bazett. Our average values are higher than Gibson's (9) general averages, but agree with his figures for athletic individuals.

The discrepancy between the small change we observed and the large ones previously reported is hard to explain. Barcroft measured the cell volume by the CO method. Bazett in most cases measured the plasma

volume by the vital red method, which is essentially the same as ours except for the kind of dye used. He also used CO in a few experiments and T1824 in others. Both calculated the blood volume from the hematocrit readings. The former of these methods gives lower results than the latter (fewer cells in the capillary blood? very rapid removal of a small part of the dye?), but the change with climate should be the same with either method, and so it is in Barcroft's and Bazett's work. It is possible that there is a sudden increase followed by an equally sudden decrease. Bazett (4) has observed some indication of this, but we found no consistent difference between our values obtained within a few days of arrival and those a month later. It is true, however, that, though some of our measurements were made within three days of arrival in Mississippi, the subjects had been exposed to a few days of hot weather on the way down and conceivably might have had an increase in blood volume which had passed off. It is more likely that individual variation plus a different sort of activity may have caused the difference. In our experiments the subjects were for the most part doing medium to hard physical work and losing weight. From the accounts of the other experiments, the subjects may have been taking life easily and gaining weight. There is another possibility, namely, that the difference in results depends upon the dye used. Unlikely as this seems it is suggested by the fact that in the two experiments in which Bazett et al. used T-1824 the increases were relatively small (8 per cent and 11 per cent), and lie within the range of our observations.

The changes in available fluid were a little larger but no more consistent. Seven individuals went down and three up, but the rises were less than the falls and the average decreased 1.17 liters, or 6.9 per cent, from the Boston level of 16.87 liters. The interstitial fluid shows an even greater drop, 1.38 liters, or 12.0 per cent, from the Boston average of 11.55 liters, but again eight decreased while two increased. It might be pointed out that one of the pair whose interstitial fluid moved in the opposite direction from the other eight was the same individual whose plasma volume decreased instead of increasing, and that the other member of the pair was a frequent visitor to Mississippi and accustomed to its climate. There is a close correlation between the weight lost by an individual and the loss of interstitial fluid. If the subjects are arranged from 1 to 10 in order of their weight loss, the order of their loss of interstitial fluid per square meter of body surface runs as follows: 1, 2, 3, 4, 6, 7, 5, 10, 9, 8. Eight is the consistently atypical individual. The "rank-order" correlation is +0.89. The loss of weight exceeded the loss of interstitial fluid by 0.6 kgm. on the average, but the individual variation was great. There was a moderate degree of positive correlation between the available fluid per square meter in the different groups and the amount of sweating during a standard

piece of work, but no correlation between the efficiency and the available fluid. The interstitial fluid appeared to be less closely correlated with the sweating than the "available fluid," which includes the blood, but the number of subjects was not sufficient to be sure of this.

There was a striking difference between the white and colored groups in the amount of interstitial fluid per square meter of body surface. The

TABLE 3

Available fluid and interstitial fluid in Boston, Massachusetts, and in Benoit, Mississippi

	AVAILABLE FLUID, LITERS			AVAILABLE FLUID, LITERS PER M². OF BODY SURFACE			INTERSTITIAL FLUID, LITERS			INTERSTITIAL FLUID, LITERS PER M². OF BODY SURFACE			RATIO OF INTERSTITIAL FLUID TO BLOOD VOLUME		
	Boston	Mississippi	Δ	Boston	Mississippi	Δ	Boston	Mississippi	Δ	Boston	Mississippi	Δ	Boston	Mississippi	Δ
A. Ten White laboratory workers															
Highest..	20.80	17.50	+2.15	10.65	9.31	+1.19	15.11	12.20	+2.36	7.75	6.49	+1.30	2.31	1.93	+0.43
Lowest...	12.50	13.35	−4.05	7.35	7.18	−2.12	7.70	8.05	−4.45	4.53	4.32	−2.24	1.38	1.31	−0.94
Average..	16.87	15.70	−1.17	8.89	8.34	−0.55	11.55	10.17	−1.38	6.03	5.40	−0.68	1.89	1.59	−0.30
	% change		−6.9	% change		−6.2	% change		−12.0	% change		−11.2	% change		−15.6
B. Seven White sharecroppers in Mississippi															
Highest..		19.30			9.95			13.03			6.72			1.79	
Lowest...		12.10			6.85			6.56			3.69			1.00	
Average..		14.81			8.19			9.43			5.20			1.51	
C. Twenty-one Colored sharecroppers in Mississippi															
Highest..		22.60			13.20			17.97			10.37			3.24	
Lowest...		15.30			8.23			9.10			5.00			1.68	
Average..		16.84			9.61			11.74			6.71			2.06	
D. One Colored servant in Mississippi															
		18.60			9.80			12.86			6.69			1.89	
E. Four Colored boys from sharecroppers' families in Mississippi															
Highest..		9.20			7.92			6.48			5.58			2.06	
Lowest...		8.80			7.15			5.36			4.47			1.81	
Average..		9.00			7.52			6.01			5.03			2.01	

Whites, both the laboratory workers while in Mississippi and the native white workmen, were about the same, but only one of them had as high a value as the average for the Negroes, and only one Negro was lower than the average for the Whites. Since the Negroes were more efficient than either group of Whites in doing the standard work, we thought that there might be a correlation between the efficiency and the amount of fluid, but, as mentioned above, there was none.

Since the blood volume went up upon going to Mississippi and the interstitial fluid went down, it was evident that the greatest change would be found in the ratio of the interstitial fluid to the blood, i.e., the ratio of the fluid outside the blood vessels to that inside them. This is given in the right-hand columns in table 3. The average decrease in this ratio was nearly 16 per cent, though again two individuals went up while eight went down.

There is a pronounced difference between the Whites and the Negroes, though the northern Whites while in the North are not far below the value for the Negroes. The colored boys showed the same ratio as the colored adults, though on account of their relatively larger surface area their interstitial fluid values per square meter were out of line with the values on the colored adults and even below the white adults.

SUMMARY

A group of ten white laboratory workers on moving to a hot, damp climate for the summer showed on the average a small increase in the volumes of both blood and plasma, both absolute and relative to body weight and to surface area. The average change was +5 per cent but the range was from −6 per cent to +12 per cent.

The interstitial fluid, defined as the fluid outside the cells and outside the blood vessels, decreased in the heat 11 per cent on the average but the range was from −34 per cent to +26 per cent.

There was no difference between the colored sharecroppers, the white sharecroppers, and the laboratory workers while in Mississippi in respect to plasma volume per unit of surface area but the interstitial fluid volume was 25 per cent higher in the Negroes than it was in the white sharecroppers or the white laboratory workers.

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RELATION OF MUSCLE ELECTROLYTE TO ALTERATIONS IN SERUM POTASSIUM AND TO THE TOXIC EFFECTS OF INJECTED POTASSIUM CHLORIDE[1]

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Previous work has shown that potassium enters and leaves the cells of voluntary muscle under varying circumstances. In this laboratory it has been shown that muscle potassium is increased in the terminal stages of adrenal insufficiency and following total nephrectomy (1). Heppel has found that feeding a diet low in potassium to young growing rats resulted in marked losses of muscle potassium (2). We have independently confirmed Heppel's observations in our study of adult rats. Prolonged tetanic stimulation of skeletal muscle has been shown by Fenn (3) to produce a loss of potassium from the muscle and Baetjer (4) has found that depriving muscle of oxygen will also decrease muscle potassium.

The present work was undertaken to determine what electrolyte changes occur in serum and muscle when potassium enters or leaves muscle cells of intact normal animals. We were also interested in determining whether the capacity of skeletal muscle to take up potassium altered the toxic effects of this cation.

EXPERIMENTAL PROCEDURES. Adult male rats were used in all experiments and were fed on Purina Dog Chow (called stock diet) except when diets with altered content of sodium and potassium were sought. The special diets had the following proportions of basic ingredients: commercial lactalbumin, 18 grams; sucrose, 25 grams; commercial dextrin, 32 grams; vegetable fat (Crisco), 22 grams; cod liver oil, 1 gram; yeast powder, 2 grams; and bone ash, 2 grams. By adding sodium chloride or potassium chloride, the "high K," "low K," and "low Na" diets were made. The various diets were analyzed for Na and K and their composition is given in table 1.

The rats continued to hold or to gain weight on each diet except that some of the heavier ones on the "low K" diet lost some weight when first put on the diet. The initial loss was either regained or further loss stopped after a few days. A few of the rats which were left on the "low K" diet

for two to three months finally became sick and lost weight. None of these animals died spontaneously although some were maintained as long as 115 days before being killed. Food and water were offered to all rats up to the time of sacrifice, except that when potassium chloride was injected, food and water were withdrawn from the cages during the period of injection.

As will be brought out in the presentation of data, a number of rats received intraperitoneal injections of potassium chloride in a solution containing 200 mM. of KCl per liter. Except in group IX, which received a single fairly large injection, the solution was given in several small injections at intervals of one to two hours starting at 9 a.m. and continuing until 5 p.m. or until the animals were killed. When injections were carried out for more than one day, the rats received none overnight, an interval of about 17 hours.

In group IV a solution containing NaCl 1.2 per cent and sodium bicarbonate 0.4 per cent was injected intraperitoneally. This solution

TABLE 1

Composition of diets by analysis

	mM PER 100 GRAMS	
	K	Na
Stock..	15.5	23.7
High K...	25	1.7
Low K..	1.6	17.7
Low Na...	16.6	1.7

was likewise given at intervals of one to two hours during a period of fasting.

All animals were anesthetized with ether before being killed by withdrawing as much blood as possible from the abdominal aorta. Tissue was removed and analyzed as described in a previous paper (5). In the tables serum Na and Cl are expressed per liter of ultrafiltrate of serum, using the dried weight for water and a Gibbs-Donnan factor of 0.96. Serum potassium is expressed per liter of serum. Tissue analyses are expressed per 100 grams of fat-free solids except potassium. In the case of this cation the tissue concentrations have been reduced to intracellular potassium by subtracting extracellular potassium. The latter value was assumed to be measured by the concentration in serum and the extracellular volume of water. Extracellular water was measured by the ratio of tissue chloride to the concentration of chloride in the ultrafiltrate of serum.

EXPERIMENTAL RESULTS. *I. Variations in muscle potassium occurring with normal serum electrolyte.* Table 2 shows that the muscle potassium

of adult rats may vary from 44 to 50 mM. per 100 grams of fat-free solids at times when the concentration of potassium in the serum is normal. Rats in groups I and V had muscle potassium values in the upper half of this range, i.e., from 47 to 50 mM. per 100 grams of fat-free solids and rats in groups II, III and IV had values in the lower half, i.e., from 44 to 47 mM. Rats in group I were fed the stock diet of Purina Dog Chow

TABLE 2

Muscle potassium accompanying normal serum potassium concentrations

GROUP	DIET	NUMBER OF RATS	SERUM			MUSCLE					
			Per liter serum ultrafiltrate		Per liter serum	Per 100 grams fat-free solids					
			Na	Cl	K	Na	Cl	K*	P	Protein	H₂O
			mM	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*grams*	*cc.*
I	Stock	13	146.9	113.0	4.0	9.99	7.2	48.8	33.4	92.3	340.5
			±0.74	±0.87	±0.21	±0.18	±0.15	±0.18	±0.35	±0.56	±1.90
II	"High K"	9	147.6	118.3	4.2	10.6	7.0	45.2	31.9	95.4	337.4
			±0.83	±1.37	±0.19	±0.33	±0.23	±0.36	±0.37	±0.56	±2.66
III	Stock	10	148.1	112.9	4.6	9.3	6.7	46.9	32.6	97.2	329.6
			±0.93	±0.88	±0.33	±0.25	±0.25	±0.33	±0.28	±0.64	±1.71
IV	Stock	4	141.2	114.5	4.8	10.9	7.6	45.9	32.6	97.0	341.8
			±2.29	±1.26	±0.26	±0.36	±0.20	±0.72	±0.34	±0.82	±2.86
V	"Low Na"	5	149.4	112.2	4.8	10.3	7.6	48.3	32.8	95.1	343.0
			±1.72	±0.08	±0.04	±0.002	±0.02	±0.51	±0.06	±0.54	±2.21

Group I. Control group.

Group II. On diet 24 to 51 days. Half of group were also given NaCl in water with the same result.

Group III. Injected with 3 to 4.6 meq. KCl per 100 grams of rat in 72 hours. Survived 18 hours after last injection.

Group IV. Injected with 4.8 to 12.1 meq. Na per 100 grams of rat in 1.2% NaCl and 0.4% NaHCO₃ solution over a period of 72 to 96 hours. Survived 18 hours after last injection.

Group V. On diet 16 days.

* Corrected for extracellular K.

All concentrations represent mean result plus or minus the standard error.

and the muscle potassium of these rats may be considered as representing the usual values for normal healthy adult rats. Rats in group V were fed a diet similar in its potassium content to the stock diet but low in sodium. Since the muscle potassium of rats in group V was similar to that of rats in group I, the decrease in muscle potassium of rats in group II, some of which were also fed a diet low in sodium, is not dependent on their low sodium intake. The fact that half of the rats in group II were

given added sodium chloride in their drinking water without producing significant differences in their muscle potassium from the other half of the group is further indication that the decrease of the muscle potassium of rats in group II to the low normal range was in some way associated with the high potassium intake in the diet. Further support for the concept that muscle potassium may actually be lowered by a high potassium

TABLE 3

Muscle potassium accompanying increased serum potassium concentrations

GROUP	DIET	NUM-BER OF RATS	SERUM			MUSCLE					
			Per liter serum ultrafiltrate		Per liter serum	Per 100 grams fat-free solids					
			Na	Cl	K	Na	Cl	K*	P	Protein	H₂O
			mM	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*grams*	*cc.*
I	Stock	13	146.9 ±0.74	113.0 ±0.87	4.0 ±0.21	9.99 ±0.18	7.2 ±0.15	48.8 ±0.18	33.4 ±0.35	92.3 ±0.56	340.5 ±1.90
VI	Stock	7	143.4 ±0.54	121.4 ±0.75	7.5 ±0.20	8.2 ±0.26	7.3 ±0.14	51.7 ±0.52	34.5 ±0.29	95.9 ±1.03	353.0 ±1.54
VII	Stock	5	145.2 ±0.86	120.0 ±1.14	5.5 ±0.23	8.7 ±0.30	7.2 ±0.19	49.5 ±0.36	33.2 ±0.18	94.8 ±1.28	346.3 ±2.82
VIII	Stock	4	143.2 ±0.81	128.0 ±1.41	14.7 ±0.37	8.3 ±0.48	9.7 ±0.43	53.1 ±0.96	32.0 ±0.59	92.9 ±1.43	359.2 ±1.08
IX	Stock	8	137.0 ±3.21	115.3 ±2.47	12.5 ±2.07	7.5 ±0.29	7.4 ±0.30	49.2 ±0.24	31.9 ±0.21	95.1 ±0.30	356.6 ±7.08

Group I. Control group.

Group VI. Injected 1.1 to 1.4 meq. KCl per 100 grams of rat 6 to 7 hours and sacrificed 15 to 30 minutes after last injection.

Group VII. Injected 1.1 to 1.3 meq. KCl per 100 grams of rat 6 to 7 hours and sacrificed 60 to 90 minutes after last injection.

Group VIII. Injected 1.5 to 3.0 meq. KCl per 100 grams of rat 8 to 29 hours and sacrificed 15 to 40 minutes after last injection.

Group IX. Injected 0.5 to 2.0 meq. KCl per 100 grams of rat in one injection and sacrificed within 30 to 60 minutes.

* Corrected for extracellular K.

All concentrations represent mean result plus or minus the standard error.

intake is found in the results observed in rats in group III. These animals were repeatedly injected intraperitoneally with small amounts of potassium chloride over a period of three or four days and sacrificed eighteen hours after the last injection. From data that will be presented in table 3 it is certain that the injected rats had high serum and muscle potassiums during the period of injections, but that during the interval between the

last injection and the time of sacrifice the muscle potassium not only returned to its original levels (47 to 50 mM. per 100 grams of fat-free solids) but actually decreased further to low normal values (44 to 47 mM.).   It is important to note that at least at the time of sacrifice the concentration of potassium in the serum of rats in group III was within normal limits. We emphasize this point because it will subsequently be shown (table 4) that decreases in muscle potassium below 44 mM. per 100 grams of fat-free solids were invariably associated with significant decreases in the concentrations of serum potassium.   The decrease in muscle potassium to low

TABLE 4

Serum and muscle electrolyte of rats with muscle potassium below normal.   Results of injecting KCl into rats with abnormally low muscle potassium

GROUP	DIET	NUMBER OF RATS	SERUM			MUSCLE					
			Per liter serum ultrafiltrate		Per liter serum	Per 100 grams of fat-free solids					
			Na	Cl	K	Na	Cl	K*	P	Protein	H₂O
			mM	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*grams*	*cc.*
I	Stock (Purina Dog Chow)	13	146.9 ±0.74	113 ±0.87	4.0 ±0.21	9.99 ±0.18	7.2 ±0.15	48.8 ±0.18	33.4 ±0.35	92.3 ±0.56	340.5 ±1.90
X	"Low K" (Control)	6	147.5 ±1.07	106 ±1.52	2.5 ±0.24	15.1 ±1.50	6.6 ±1.80	37.6 ±1.88	31.6 ±0.49	96.6 ±1.97	332.8 ±2.69
XI	"Low K" (Injected KCl)	6	145.2 ±1.07	119.8 ±3.00	8.3 ±1.79	11.3 ±0.67	7.5 ±0.44	46.9 ±0.35	32.6 ±0.35	95.5 ±0.62	338.3 ±2.20

"Low K" X averaged 57 days on diet.

"Low K" XI averaged 47 days on diet.   Injected 2.6 to 3.7 meq. KCl per 100 grams of rat over a period of 26 to 48 hours.   Survived 1 to 17 hours after the last injection.

* Corrected for extracellular K.

All concentrations represent mean result plus or minus the standard error.

normal levels (44 to 47 mM.) can be produced by other methods than increasing the intake of potassium.   Similar decreases in muscle potassium were observed in rats in group IV that were repeatedly injected intra-peritoneally with relatively large amounts of a mixture of NaCl and Na HCO₃ over a period of three to four days.

The fact that the concentrations of serum potassium in table 2 do not show significant differences does not preclude the possibility that as the muscle potassium varies between 44 and 50 mM. per 100 grams of fat-free solids there may be changes in the concentration of serum potassium. Subsequent tables show that fluctuations in muscle sodium and potassium

may be quite rapid following significant alterations in the concentration of serum potassium. The data in table 2 merely indicate that at the time of sacrifice the concentrations of potassium in the serum were normal. In addition to finding normal concentrations of potassium in the serum in table 2 we observed that the concentrations of sodium and chloride in the serum likewise were within normal limits except for a somewhat high chloride in the serum of rats in group II and a low sodium in rats in group IV. It was also observed that the muscle sodium and chloride of rats in all groups in table 2 did not differ significantly, giving further support to the concept that the range of muscle potassium between 44 and 50 mM. per 100 grams of fat-free solids may be looked upon as a range of normal physiological adjustment of muscle electrolyte.

Balance experiments were conducted in a few rats in groups III and IV and indicated that the low muscle potassium of these rats could be explained by potassium diuresis.

II. Variations in muscle potassium accompanying increased concentration of serum potassium. The analyses in table 3 show that while potassium probably readily enters muscle cells, it is difficult to produce sustained high values. In group VI the rats were given small doses of KCl intraperitoneally each hour for 6 to 7 hours and then killed 15 to 30 minutes after the last injection. All of these animals showed high muscle potassium and slightly low muscle sodium while serum potassium was moderately high. However, if the animals were treated in the same way but killed after 60 to 90 minutes, as in group VII, muscle potassium returned to the normal level and serum potassium was only slightly elevated. None of the animals in groups VI and VII showed symptoms of potassium poisoning.

If larger doses of potassium are given, symptoms of potassium poisoning develop. These symptoms appear within 10 to 20 minutes and if death does not occur in about 50 minutes after an injection recovery gradually ensues. Group VIII represents animals obviously about to die spontaneously as the result of repeated large doses of potassium chloride. While the muscle potassium is higher than in group VI, the striking difference is in the high serum potassium. However, single large doses of potassium chloride in group IX did not raise muscle potassium to abnormal levels although serum values are at levels known to produce electrocardiographic changes and almost as high as in group VIII.

Attention is directed to the low values for muscle sodium in all rats injected with potassium chloride.

III. Variations in muscle potassium produced by diets low in potassium. In contrast to the difficulty in sustaining muscle potassium above the normal level is the readiness with which muscle rendered deficient in potassium retains potassium injected intraperitoneally. In table 4,

group X shows the muscle values in rats fed the diet low in potassium. In each of the rats of this group both the serum and muscle potassiums are abnormally low, while the muscle sodium is obviously high. Since muscle chloride is not high, the increase in muscle sodium must be intracellular rather than extracellular. The changes in muscle sodium and potassium become more marked the longer the rats are kept on the diet deficient in potassium. The lowest value for muscle potassium (29 mM.) was obtained on a rat kept on the diet for 115 days.

Before injection with potassium chloride group XI was treated like group X and undoubtedly had muscle potassiums of the same order of magnitude. The higher muscle potassium in group XI than in group X was brought about by the injection of potassium chloride and the potassium which went into the muscle cells remained in the muscle at least 17 hours. This is contrary to the experience in groups VI, VII and III, when potassium rendered abnormally high returns to high normal levels within 60 to 90 minutes after the last injection and to low normal levels, i.e., 45 mM. per 100 grams of fat-free solids, if 18 hours elapses after the last injection.

By balance experiments it was found that injecting potassium chloride leads to a retention of potassium in rats previously fed a diet low in potassium while a negative balance was found by a similar injection in rats fed a normal diet.

It will be noted that muscle sodium returned toward normal when muscle potassium was raised in group XI.

IV. Interchangability of muscle sodium and potassium. As was shown by Heppel (2) and pointed out by us in the previous paragraphs, there is evidence of a reciprocal relationship between muscle sodium and potassium. Since from quantitative considerations extracellular potassium cannot be involved in this interrelationship, the values involved must be intracellular sodium and potassium. Since sodium is both intracellular and extracellular, a first approximation of the value for intracellular sodium was calculated as follows:

$$\frac{(Cl)_t}{[Cl]_s} = (H_2O)_e$$

$$(Na)_t - [Na]_s \times (H_2O)_e = (Na)_i$$

in which $(Cl)_t$ represents total tissue chloride; $[Cl]_s$ is the concentration of chloride in an ultrafiltrate of serum; $(Na)_t$ is sodium of tissue: $[Na]_s$ is the concentration of sodium in an ultrafiltrate of serum; $(Na)_i$ is intracellular sodium and $(H_2O)_e$ is extracellular water. It must be borne in mind that, since some muscle chloride is not diffusible (6), the extracellular water calculated by the ratio $\frac{(Cl)_t}{[Cl]_s}$ is somewhat too large. Hence intra-

cellular sodium, $(Na)_i$, is too small. The error is probably fairly constant although its exact magnitude is not known. This error accounts for the appearance of a negative value for intracellular sodium in some instances.

Figure 1 shows the relationship between this calculated intracellular sodium and muscle potassium. It will be seen that there is a good inverse relationship. Apparently for each 2 mM. of potassium that leaves the cells, about 1 mM. of sodium enters the cells. The negative values indicate that the calculated value for intracellular sodium is about 2 mM.
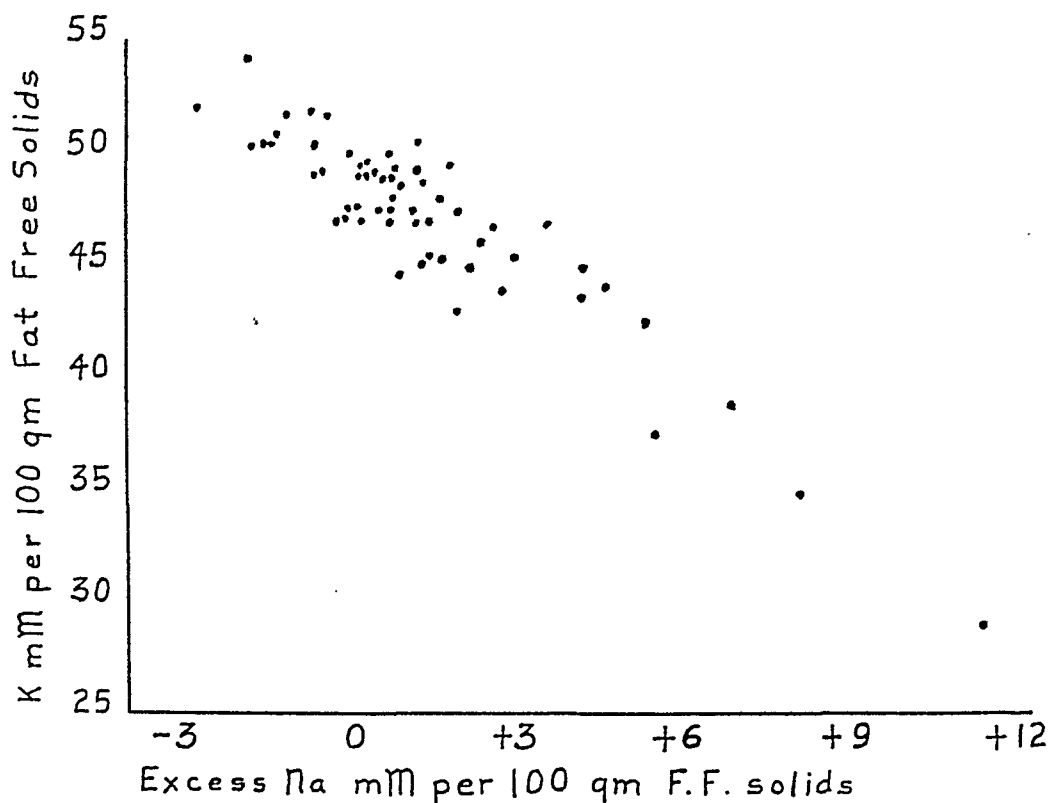
Fig. 1

too small. This assumption fits in with the evidence that about 1 mM. of chloride is associated with connective tissue (7) and is not diffusible (6).

V. *The relation of muscle potassium to the toxic effect of injected potassium.* In all groups of rats injected with KCl toxic effects were obtained in certain instances. With large doses of KCl the rats began to breathe rapidly within ten to fifteen minutes; this dyspnea rapidly increased and the animals became disinclined to move when disturbed; terminally they became cyanotic and pale. Death occurred in 5 to 40 minutes after the onset of symptoms. Apparently if the dose of KCl did not kill within 40 minutes, recovery gradually occurred. At death the typical dilated heart

of potassium poisoning was observed. From observation it was possible to predict the outcome and grade the degree of toxic effect. Table 5 was arranged to demonstrate the relation between the toxic effect of the injected KCl and the potassium level in the muscle and serum.

In general the severity of the toxic symptoms seems to be directly associated with the level of serum potassium. When the serum potassium was less than 10 mM. per liter, no symptoms or only a moderate degree of toxicity were noted at the time of sacrifice. In all instances in which

TABLE 5

Relation of toxic effect of injected KCl to muscle potassium

DIET	NUMBER OF EX- PERIMENT	K AB- SORBED* FROM PERITO- NEUM	PERIOD OF SURVIVAL	CLINICAL CONDITIONS	SERUM POTASSIUM	MUSCLE POTASSIUM
		meq./100 grams of rat	*minutes*		*meq./liter of serum*	*meq./100 grams fat-free solids*
"Low K"	6	0.75	40 (killed)	Not sick	5.9	43.7
"Low K"	9	0.77	55 (killed)	Not sick	7.9	42.0
Stock	7	0.39	45 (killed)	Moderately sick	8.7	49.0
Stock	8	0.50	55 (killed)	Moderately sick	8.7	50.1
Stock	14	0.52	40 (killed)	Very sick; dyspneic, breathing fast, apathetic	13.6	49.6
Stock	11	0.66	40 (killed)	Very sick; dyspneic, breathing fast, apathetic	14.2	49.6
Stock	12	0.89	35 (died)	Convulsions	19.9	49.8
Stock	13	1.28	17 (died)	Convulsions	21.8	49.1
"Low K"	10	1.17	48 (died)	Convulsions	21.3	44.3
"Low K"	15	1.39	55 (died)	Convulsions	20.5	37.1

* K absorbed equals the amount of K remaining in peritoneal fluid as actually determined at times of sacrifice subtracted from the measured amount of K injected intraperitoneally.

serum potassium was as high as 20 mM. per liter, spontaneous death occurred or was about to occur. As shown by experiments 6 and 9, rats on a "low K" diet were strikingly less susceptible to the toxic effect of KCl than rats on the stock diet (expts. 7 and 8) despite the fact that a larger dose of KCl was absorbed from the peritoneal cavity. It is noteworthy that, despite the absorption of more KCl, the muscle and serum potassium are lower in experiments 6 and 9 than in experiments 7 and 8. In the case of experiments 14 and 11, the increased toxicity on smaller doses of KCl is marked. Although the rats on "low K" diet are more

resistant, it is possible to produce fatal effects as is shown in experiments 10 and 15. The difference in reaction in this case is brought out by the longer period of survival. It will be noticed that low muscle potassium did not protect the rats from the effects of the rapidly rising serum K which had reached 20 mM. at the time of death.

DISCUSSION. The data have been presented chiefly from the point of view of the factors which bring about variations in muscle potassium and the effects of these variations on susceptibility to the toxic manifestations following injection of potassium chloride. The other tissue analyses are included to demonstrate that the variations in muscle potassium are not necessarily accompanied by changes in the other factors excepting intracellular sodium. There are significant variations in protein per 100 grams of fat-free solids. Presumably increase in protein indicates decrease in some other fat-free solids rather than increase in protein. Changes in glycogen could account for the alterations in protein per 100 grams of fat-free solids shown in the tables. Since the changes in potassium are not accompanied by constant changes in protein, variations in muscle potassium are not accounted for by variations in non-protein fat-free solids (glycogen?). Since phosphorus is fairly constant, variations in muscle potassium and intracellular sodium do not apparently alter the cellular content of phosphorus compounds.

The variations in tissue water are in part accounted for by variations in the concentration of extracellular sodium and in part by variations in sodium and potassium of the muscle. Nevertheless the relationships between various factors of the tissue which might express osmotic pressure and the concentration of sodium plus potassium in the ultrafiltrate of plasma are quite variable. This result is explained in part by the fact that many of the analyses represent conditions in serum and muscle at a time when rapid adjustments are being made. For instance, evidences of shift of both water and sodium out of the cell are obtained within fifteen minutes after the injection of potassium chloride into the peritoneal cavity. Also within sixty to ninety minutes after muscle potassium has been elevated to abnormally high values, the concentration in muscle is restored to normal (see groups VI and VII). Hence while muscle water undoubtedly tends to increase when sodium and potassium are retained in muscle (6), the present data do not give a satisfactory expression of these relationships.

Additional observations support the idea that muscle potassium ranging from 44 to 47 mM. per 100 grams of fat-free solids must be regarded as normal although the usual values vary from 47 to 50 mM. For example, occasionally rats on a normal diet and otherwise not subjected to any unusual strain show values for muscle potassium at the lower level. The same observation has been made on cats (6). Furthermore, it is rather

easy to produce in a short time the lower concentration of muscle potassium. When values below 44 mM. actually have been produced, abnormally low concentrations of potassium in serum have almost invariably been encountered. Similarly concentrations of muscle potassium above 50 mM. always are accompanied by high concentrations of potassium in serum. Nevertheless there is no obligate relationship between the concentration of potassium in serum and the concentration of potassium in muscle.

The inverse relationship between intracellular sodium and potassium that has been shown to exist in the present study applies equally well to animals in adrenal insufficiency and in nephrectomized rats (1). The exchange of potassium for sodium in the muscle cell in these animals does not cover so wide a range as we found in the present study since the variation in muscle potassium was not so extreme. The replacement of potassium by sodium in the muscle cells has also been demonstrated in this laboratory to occur in rats injected with desoxycorticosterone (8). So far we have not been able to ascertain any conditions where this inverse relationship does not exist providing the exchange is large enough to be detected by the methods used.

Previous work has shown that evidences of potassium poisoning can be closely correlated with the level of serum potassium (9). In turn the concentration of serum potassium has been shown to affect cardiac rhythm, contraction and tone (9). The present experiments are entirely in accord with previous work emphasizing the importance of the concentration of serum potassium in determining the onset of potassium intoxication. However, the present data demonstrate, in addition, that low levels of muscle potassium delay the onset of toxic elevation of serum potassium and permit larger doses of potassium to be given without evidences of potassium poisoning. The data show why adrenalectomized animals that manifest defective renal excretion of potassium and tend to have high muscle potassium are peculiarly susceptible to the toxic effect of this cation.

SUMMARY

The usual range of muscle potassium in adult rats is 47 to 50 mM. per 100 grams of fat-free solids. Muscle potassium levels as low as 44 mM. may occur even though the concentration of potassium and sodium in the serum and the amount of sodium in the muscle remain normal.

Increases in muscle potassium above 50 mM. produced by injecting KCl are transitory in normal intact animals and return to high normal levels (49 mM.) within sixty to ninety minutes after the last injection and to low normal levels (45 mM.) eighteen hours after the last injection. Such transitory increases in muscle potassium are accompanied invariably by a shift of sodium out of the cell and have not been demonstrated except

in the presence of an increased concentration of serum potassium. With the fall in muscle potassium which follows the transitory rise, the serum potassium concentration and muscle sodium return towards normal levels. Significant increases in muscle potassium occurred without evidence of potassium poisoning in animals in which the serum concentration of potassium was not elevated above 10 meq. per liter.

Increases in muscle potassium in animals whose muscle has previously been rendered deficient in potassium (i.e., from 29 to 44 mM. per 100 grams of fat-free solids) remain fixed at least for seventeen hours after the last injection of KCl. The tolerance of animals with low muscle potassium to the toxic effects of injected potassium is appreciably greater than the tolerance of animals with normal muscle potassium. It is suggested that the slower rise in serum potassium concentrations in the former is due to the greater capacity of the skeletal muscle to take up potassium from extracellular fluid. The toxic effects of injected potassium are directly related to the elevation in concentration of serum potassium and only indirectly to that of muscle potassium.

Within the range of muscle potassium between 29 and 55 mM. per 100 grams of fat-free solids found in these experiments there is a reciprocal relationship between muscle potassium and the intracellular sodium of the muscle. It was found that about 1 mM. of sodium may be interchanged for 2 mM. of potassium.

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THE ACTION OF HEPARIN, SERUM ALBUMIN (CRYSTALLINE), AND SALMINE ON BLOOD-CLOTTING MECHANISMS (IN VITRO)

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The two phases of blood-clotting, viz. 1, formation of thrombin from prothrombin, and 2, thrombic conversion of fibrinogen into fibrin, may be studied quantitatively *in vitro* (1).   A rational approach to the experimental analysis of the mode of action of any anticoagulant is to observe its behavior in the respective phases of such artificially isolated systems. This type of approach led Howell (2) to separate clot-inhibitors into *a*, *antiprothrombic*, if they act on the first phase, and *b*, *antithrombic*, if the action involves the second phase.   We shall adhere to these general terms and, again following Howell, shall designate as *thromboplastic* any agent which assists the first phase reaction.   The term *fibrinoplastic* will be used for any assistance in the second phase.

Since serum albumin and salmine (a basic protamine, which combines with heparin (3), after the manner of heparin-protein compounds (4)) have been reported to be anticoagulants, each showing some interesting relationships to the action of heparin, the following investigation was undertaken in an attempt to analyse the fundamental mechanisms involved.

Experimental technique.   Methods for preparing the various clotting agents (prothrombin, cephalin, $CaCl_2$, thrombin, fibrinogen) have previously been detailed (1), together with the technique for the clotting-tests and for the study of the first phase (prothrombin activation).   The crude *thromboplastin* used for most of the present experiments consisted of an aqueous extract (diluted) of fresh or frozen dog brain or of the acetone-precipitated, air-dried residue of similar extracts.   *Crude plasma "albumin"* consists merely in the dialysed plasma residue after removal of fibrinogen and globulins by half-saturation with $(NH_4)_2SO_4$.

We are indebted to 1, Dr. C. H. Best and the Connaught Laboratories (Toronto) for a purified lung-*heparin*, assaying 110 "units" per mgm.; 2, Dr. T. L. McMeekin (Harvard) for the *crystalline serum albumin*; 3, Messrs. E. R. Squibb and Sons (Brunswick, N. J.) for the *salmine* (-*sulfate*), and 4, Dr. J. H. Northrop (Rockefeller Institute) for the *crystalline trypsin*.[1]

pH was controlled (unless otherwise stated) with the glass electrode (Beckman pH meter), routinely to pH = 7.25.   The heparin (1:1000 soln.) had an initial pH

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[1] *Crystalline trypsin* is now available commercially (Plaut Research Laboratories, Lehn & Fink Products Corp., Bloomfield, N. J.).

of 4.1, well buffered; the salmine sulfate (1:200 soln.) was very poorly buffered at pH = 5.1; a 1:10 dilution of McMeekin's crystalline serum albumin (before dialysis) gave a pH of 5.85, also poorly buffered.

Volumetric pipettes were used for accurate measurement of the quantities of the various reagents and all clotting-tests (2nd phase) refer to time of appearance of first visible strands of fibrin in mixtures held in small tubes in a thermostat water-bath at 38°C.

Plasma experiments. Since the action of the cited anticoagulants on whole blood and plasma has been studied repeatedly by a number of investigators, the data of table 1 serve principally to show modifications of the inhibitory phenomena in the presence of various thromboplastic agents (cephalin: crystalline trypsin). The ability of trypsin (thrombo-

TABLE 1

Effects of heparin and salmine on clotting of recalcified citrated dog plasma, in presence of various thromboplastic agents

Clotting-times (seconds), at 38°C., for 1 cc. plasma + 0.25 cc. N/10 $CaCl_2$ + 0.75 cc. of thromboplastic and other agents (quantities expressed in *milligrams*).

NUM-BER	INHIBITOR	(Ca, ONLY)	CEPHALIN (0.5)	XTAL. TRYPSIN (0.05)	TRYPSIN (0.05) + CEPHALIN (0.5)
1		140 sec.	45 sec.	110 sec.	15 sec.
2	Heparin (0.1)	510 sec.	240 sec.	270 sec.	20 sec.
3	Heparin (0.5)	∞	∞	∞	290 sec.
4	Salmine (0.025)	400 sec.	100 sec.	120 sec.	15 sec.
5	Salmine (0.25)	1040 sec.	180 sec.	240 sec.	25 sec.
6*	Salmine (2.5)	>1 hr.	c 40 min.		
7	Hep. (0.1) + Salm. (0.025)	240 sec.	60 sec.	185 sec.	16 sec.
8	Hep. (0.5) + Salm. (0.025)	∞	310 sec.		
9	Hep. (0.5) + Salm. (0.25)	270 sec.	105 sec.	∞	22 sec.
10*	Hep. (0.5) + Salm. (2.5)	>6<16 hrs.	>2<6 hrs.		

* Flocculated.

plastic enzyme), especially in conjunction with added cephalin, to overcome most of the inhibitory effect of heparin (5) is again demonstrated. The same effect is shown also for salmine and, even more strikingly, for salmine *plus* heparin. pH was not controlled in these experiments, but plasma is well-buffered.

The heparin-salmine antagonism (3) is confirmed.

Serum albumin experiments. Tests with crude plasma "albumin." 1. *First phase* reactions were not studied with this material. 2. *Second phase* studies confirmed and extended the observations previously reported (5). There was usually an insignificant retardation of the clotting of fibrinogen by thrombin, in the presence of the "albumin" preparation, even when pH was controlled (7.25). Sometimes, however, a slight

fibrinoplastic effect (clot-acceleration) occurred instead, especially with the more dilute preparations.   Table 2, no. 2, is typical of several experiments designed to test Quick's (6) assertion of a "progressive" antithrombic effect.   On incubating a *stable* thrombin (no. 1) for an hour at room temperature (24°C.) with the "albumin" preparation, there was seen only a minor and irregular effect on thrombic activity (tested at the cited intervals), not at all like the marked "progressive" inactivation described by Quick.

As before (5), however, the striking synergism with heparin (Quick) is again manifested (no. 4).

Tests with crystalline serum albumin.   1. In the *first phase:* During the formation of thrombin from prothrombin, added crystalline serum albumin was consistently *thromboplastic* throughout a number of experiments of the type illustrated in table 3.   Furthermore, it always lessened the

TABLE 2

Effects of incubation (with thrombin) on the antithrombic action of crude plasma "albumin" and heparin (1:5000)

Thrombin (1 volume) + anticoagulant ($\frac{1}{4}$ volume, each, suitably diluted): incubated, at 24°C., for times (minutes) indicated.   Clotting-times (seconds), at 38°C., for 1 cc. fibrinogen + 0.5 cc. mixture.

NUM-BER		$\frac{1}{4}$ MINUTE	5 MINUTES	15 MINUTES	30 MINUTES	60 MINUTES
1	(Control)	18 sec.	18 sec.	18 sec.	18 sec.	18 sec.
2	Albumin	18 sec.	10 sec.	12 sec.	16 sec.	33 sec.
3	Heparin	25 sec.	25 sec.	25 sec.	25 sec.	25 sec.
4	Alb. + Hep.	39 min.	>4 hr.	>4 hr.	>4 hr.	>4 hr.

antiprothrombic action of heparin, in parallel tests, whether a weak brain thromboplastin or cephalin was the main thromboplastic agent used.

2. In the *second phase:* The "immediate" effect of adding crystalline albumin to fibrinogen-thrombin mixtures showed the same minor variability on clotting-time as in the case of the corresponding experiments with crude "albumin."   Thus, there was a few seconds' lengthening of the clotting-time in most instances, but an occasional slight acceleration occurred in some experiments.

Varying the salt content by dialysis (as compared with simple dilution) or by the use of 0.85 per cent NaCl (*vs.* distilled water) for several dilutions, gave no indication of any relationship of these effects to the salt content.   Simple pH effects were ruled out by having the albumin, thrombin, and fibrinogen all at the same pH (7.25).   Controlled modification of pH, in both first and second phase reactions, will be discussed in a separate section.

TABLE 3

Effects of crystalline serum albumin and heparin on the activation of prothrombin to thrombin

Thrombic mixture (T) = 4.0 cc. prothrombin + 0.25 cc. N/10 $CaCl_2$ + 0.75 cc. thromboplastic agent (A, dilute brain extract; B, cephalin) *plus* anticoagulant, incubated, at 10°C., for time (minutes) indicated.   Clotting-times (seconds) for 1 cc. fibrinogen + 0.5 cc. T, at 38°C.   pH = 7.25.

NUM-BER		5 MINUTES	15 MINUTES	30 MINUTES	60 MINUTES	120 MINUTES
		sec.	sec.	sec.	sec.	sec.
	A. Brain thromboplastin					
1	(Control)	40	15	12	12	12
2	Crystalline albumin	18	15	15	17	18
3	Heparin	262	72	29	21	19
4	Albumin + heparin	200	33	24	21	21
	B. Cephalin (weak)					
5	(Control)	145	115	85	43	
6	Crystalline albumin	85	45	25	15	
7	Heparin	2700	2370	1860	960	
8	Albumin + heparin	1530	1580	1320	750	

TABLE 4

Effects of incubating thrombin with crystalline serum albumin (McMeekin's, diluted 1:20) and heparin (1:1000)

Clotting-times (seconds) at 38°C., and pH = 7.1, for 1 cc. fibrinogen + 0.5 cc. anticoagulant *plus* thrombin mixtures.   Mixtures, consisting of 1 volume thrombin solution + 1 volume of anticoagulant (diluted, where necessary), held at 24°C. and pH = 7.1, for times (minutes) indicated.

NUM-BER		½ MINUTE	5 MINUTES	15 MINUTES	30 MINUTES	60 MINUTES
		sec.	sec.	sec.	sec.	sec.
	A. Stable thrombin:					
1	(Control)	18	18	18	18	18
2	Albumin*					
3	Heparin (1:1000)	24	26	26	26	26
4	Alb. + Hep.	27	42	42	42	42
	B. Slightly unstable thrombin:					
5	(Control)	14	14	14	15	18
6	Albumin (1:20)	21	22	25	29	36
7	Heparin (1:1000)	48	78	115	160	185
8	Alb. + Hep.	73	103	138	165	205

* Tests with albumin alone are lacking in series A, but a test made at the same time, on another thrombin, showed the usual *slight* (few seconds) initial "anti-thrombic" effect, and in some other tests this showed no increase in 1 hr.

Incubation of thrombin with crystalline serum albumin for an hour gave no evidence of any significant "progressive" effect.

In sharp contrast to the crude "albumin," there was *no* sharp increase in antithrombic effect with heparin *plus* the pure albumin. In cases where the albumin was slightly antithrombic, there was a minor additive effect superimposed on the similar heparin action. When the albumin was fibrinoplastic, the heparin effect was reduced. Besides being of small magnitude, the aforementioned antithrombic addition effect was *non-progressive* in the case of stable thrombins (4, no. 4).

Effect of stability of thrombins. Some thrombin preparations are much less stable than others, even when held at low temperatures (10°–15°C.). We have observed that a thrombin, which tends to undergo spontaneous inactivation, even if the rate of deterioration is so slow that it barely shows up within the hour period of the accompanying tests (see control, no. 5), does give progressively longer clotting-times in the presence not only of albumin, but also with heparin alone and, most markedly, with heparin *plus* albumin. Obviously, this is not a synergism of the albumin and heparin effects, but a modification of the thrombin inactivation process. The inactivation phenomenon will be the subject of a separate communication (7).

Experiments with salmine. 1. In the *first phase*: The presence of salmine-sulfate (at same pH as the other reagents) resulted in a marked *antiprothrombic* effect in the early phases of prothrombin activation. This was found consistently, except in a few cases (upon which we place no emphasis) where the thromboplastic agent was omitted. As usual in these cases, the activation by calcium alone was slow and poor. The anomalous thromboplastic action of salmine was noted in the early phases (in some experiments even more conspicuously than in the example cited). In most cases, the antiprothrombic effect developed later, although rather irregularly, as in the cited experiment (no. 8).

Heparin (v. infra) is also an antiprothrombin, but the data of table 5 clearly reveal the *antagonism* between salmine and heparin in this respect. With the right quantitative mixture (no. 6), the antiprothrombic effects of the two agents neutralize each other completely and the activation curve accurately reproduces that of the control (no. 1).

2. In the *second phase*: Salmine was consistently fibrinoplastic in the thrombin-fibrinogen reaction and sometimes quite strikingly so. The action was quantitative, disappearing on sufficient dilution. It was non-progressive. These facts are brought out in table 6.

The relations of salmine and heparin effects in the second phase are shown in table 8, in the section dealing with pH effects. It is seen, throughout the pH range, that salmine *immediately* overcomes most of the minor direct antithrombic action of heparin.

Experiments with heparin. The data on the unequivocal *antiprothrombic* action of heparin in the first phase and the very minor immediate (non-

progressive) *antithrombic* effect in the second phase (at all pH levels tested) are included in the accompanying tables. The experimental results also show that the antiprothrombic action of heparin is neutralized in a quantitative manner by salmine, but is only slightly reduced by crystalline serum albumin. In the second phase, the minor antithrombic

TABLE 5

Effects of salmine (-sulfate) and heparin on activation of prothrombin to thrombin

Thrombic mixture (T) = 4 cc. Prothrombin + 0.25 cc. dil. brain extract (nos. 1-6 only) + 0.25 cc. N/10 CaCl₂ + inhibitors (quantities expressed in milligrams) to total volume = 5 cc. Incubation (at 25°C.) for times indicated (minutes). Clotting-times (seconds) for 1 cc. fibrinogen + 0.5 cc. T, at 38°C. pH = 7.25.

NUM-BER		2 MINUTES	5 MINUTES	15 MINUTES	20 MINUTES	60 MINUTES
		sec.	sec.	sec.	sec.	sec.
1	(Ca + brain extr.)	7	5	5	6	8
2	Salmine (0.25)	3000	360	20	7	7
3	Heparin (0.05)	9	6	6	8	8
4	Heparin (0.25)	120	16	12	11	11
5	Salm. (0.25) + Hep. (0.05)	280	90	11	7	9
6	Salm. (0.25) + Hep. (0.25)	7	5	5	6	8
7	(Ca, alone)		1800	1800	900	240
8	Salmine (0.25)		695	1920	1500	1320

TABLE 6

Effects of varying amounts of salmine (-sulfate) incubated with thrombin, to test action on the second phase of clotting

Thrombic mixture (T) = 2 volumes thrombin + 1 volume salmine (cited strengths), incubated, at 24°C., for times (minutes) indicated. Clotting-times (seconds), at 38°C., for 1 cc. fibrinogen + 0.5 cc. T. pH = 7.25.

NUM-BER		1 MINUTE	5 MINUTES	15 MINUTES	30 MINUTES	60 MINUTES
		sec.	sec.	sec.	sec.	sec.
1	(Control)	22	21	22	22	26
2	Salmine (0.02 per cent)	20	19	20	20	32*
3	Salmine (0.1 per cent)	18	17	18	18	20
4	Salmine (0.5 per cent)	14	14	14	14	15

* Flocculation in mixture.

action of heparin is largely overcome by salmine, but is usually slightly increased by albumin.

When there is some inactivating process (7) in the thrombin preparation itself, this action (table 4) seems to be speeded up by heparin or albumin or both (additively). The "progressive" effect (cf. 6) is that of the throm-

bin-inactivator (*progressive* antithrombin (7)) and not of the heparin or albumin per se.

Fisher (8), in experiments on "genuine" (native) hen plasma, alleged a slight acceleration of clotting (in presence of tissue extract) when very small concentrations of heparin were added. This we have been totally unable to confirm with our reagents.

e.g. Using same reagents and test conditions as in data of table 7, plasma (dog, citrated) was clotted (by Ca + thromboplastin) in the presence of a series of heparin dilutions. The anticoagulant effect diminished steadily down to the absence of action finally obtained, as seen in the last four tests, viz.

heparin (mgm. *per test*) = $\frac{1}{160}$, $\frac{1}{320}$, $\frac{1}{640}$, $\frac{1}{1280}$;
respective clotting-times = 19, 18, 17, 17 sec.:
control (with dist. water) = 17 sec.

TABLE 7

Effect of pH on clotting of plasma, with and without heparin (1:1000) and salmine (1:100)

Samples of citrated, Berkefelded, dog plasma, diluted with an equal volume of dist. water, were brought to pH values cited. Clotting-times (seconds) at 38°C. for 1 cc. plasma + 0.25 cc. anticoagulant + 0.25 dilute brain thromboplastin + 0.25 cc. N/8 $CaCl_2$.

pH	CONTROL	HEPARIN	SALMINE (SECONDS)	CONTROL
	sec.		*flocc.*-agglut.*	*sec.*
6.0	47	Flocc. 23½ min.	60– 90	47
6.5	22	Flocc. clot 4½ min.	45– 60	21
7.0	19	130 sec.	40– 50	17
7.5	16 (16)	100 (98) sec.	35– 47	15½
8.0	15	78 sec.	40– 50	14
9.0	17 (17)	56 (57) sec.	45– 65	15
10.0	50	165 sec.	100–200	48

* Flocculation preceded agglutination in the salmine experiments: the latter is the closer approximation to a true clot.

The above-described antiprothrombic and antithrombic effects of heparin in the isolated clotting systems also diminish steadily to a vanishing point with successive dilutions of the heparin. Concentrations which have lost the ability to affect the second phase are still strongly antiprothrombic in the first phase, if cephalin (or very weak thromboplastin) is used (9).

Effects of pH. A. *On clotting of whole plasma.* In the data of table 7, the clotting-times (38°C.) of recalcified citrated plasma (with added thromboplastin) are studied at various pH levels in the range 6.0–10.0. The effects of heparin and salmine are also included. Both are inhibitory throughout the pH range tested. In the controls the optimum pH is definitely on the alkaline side of neutrality, in the neighborhood of pH = 8.0. The anticoagulant action of heparin is minimal at pH = 9.0. The

sharp increase in its inhibitory action below pH = 6.5 is accompanied by flocculation. Salmine produces a flocculation which develops in the plasma at all pH levels approximately at the times noted. With the onset of clotting the flocculation becomes coarse and agglutinative and only an approximate timing is possible. There is a suggestion that the pH optimum shifts slightly toward the acid, but the data are not sufficiently reliable to establish the optimum within 0.5 to 1.0 pH unit. The salmine tests were performed several hours later than the heparin series and the controls have altered very slightly in the direction of a somewhat more rapid clotting towards the alkaline side. This supports a finding which we have frequently experienced, both with plasma and pro-

TABLE 8

Effect of pH on clotting of fibrinogen by thrombin, in presence of crystalline serum albumin, salmine and heparin

Clotting time (seconds) at 38°C. and cited pH values, for 1.0 cc. fibrinogen + 0.5 cc. anticoagulant + 0.5 cc. thrombin. Amount of anticoagulant in each clotting test is expressed in milligrams, except crystalline albumin, which is measured in cubic centimeters of a 1:20 dilution (not dialysed) of the turbid suspension supplied by Dr. T. L. McMeekin. The last two experiments were made several hours after the first four series.

NUM-BER			10.0	9.0	8.0	7.0	7.0	6.0
				sec.	sec.	sec.	sec.	
1	(Control)		Trace, 4 hours	15	10	12	15	110 sec.
2	Albumin (0.25)		No clot	20	15	16	21	360 sec.
3	Salmine (0.25)		9 sec.	3	6	9	12	40 sec.
4	Heparin (0.25)		Trace, 6 hours	30	25	46	60	Turbid (no clot)
5	Hep. (0.25) + Alb. (0.25)		No clot	43	33	50	75	Fine flocc. (10 min.)
6	Hep. (0.25) + Salm. (0.25)		Trace, 6 hours	26	20	19	30	70 sec.

thrombic systems, viz., that thrombic activity may be enhanced after a few hours' keeping.

B. *In the second phase.* In the tests of table 8, the individual reagents were brought separately to each pH value and rapidly mixed and held at 38°C. The controls show a definite optimum, again close to pH = 8.0 although it is not defined by these experiments more closely than ±1 pH unit. There is a falling off of thrombic activity toward both the alkaline and the acid side of the optimum.

The *fibrinoplastic* action of salmine (no. 3) is shown throughout the pH range (6.0–10.0) and is most striking at the highly alkaline pH = 10, where clotting is all but inhibited in the control (and other) tests. In the data cited, the pH optimum is shifted by salmine some 1–2 pH units toward the alkaline side.

The minor *antithrombic* action of crystalline serum albumin has the same pH optimum as the control.

The similar weak *antithrombic* action of heparin also adheres to the same optimum, and this is true also of the heparin *plus* albumin. There is apparently a slight shift to the acid of the pH optimum in the case of heparin + salmine. There is no doubt that heparin has overcome the tendency of salmine to shift the optimum pH toward the alkaline side. With the reciprocal neutralization of the heparin and salmine effects, the normal (control) clotting-times are restored (approximately).

C. *In the first phase.*

Conditions for studying the effects of pH on antiprothrombic reactions are undoubtedly complicated by effects of pH (alone) on the prothrombin $\rightarrow$ thrombin reaction as well as by an influence on thrombin destruction which, at least in the later phases, seems to go on simultaneously with thrombin formation at the more acid pH levels. The method of study consists in bringing the prothrombin (alone, and with added anticoagulant) to the desired pH, then adding the thromboplastic agent and $CaCl_2$ (which tests showed to be without effect on the pH of the mixture) and incubating, at low temperature, in the usual way. The actual clotting test is performed at 38°C. by adding 0.5 cc. of thrombic mixture to 1.0 cc. fibrinogen, buffered with 1 per cent sod. barbital (at pH = 7.0 in the experiments cited in table 9, but at a somewhat more alkaline pH in other tests, confirmatory of these data). Controls at each pH are to be compared with the activation curves in the presence of the antiprothrombic agent. Preliminary tests proved that the thrombic mixture (sans Ca) at pH = 10 shifted the pH of the buffered fibrinogen less than 0.2 pH unit (to alkaline side) while the mixture at pH = 5.0 produced a shift of less than 0.35 pH unit (to acid side). These negligible changes ensure that the pH variations in the thrombic mixtures are without influence of the type studied in the preceding section.

Since the prothrombin and salmine solutions were salt-poor and feebly buffered, further control tests were made on the actual thrombic mixtures to see if the pH values were maintained. At the extremes, there was only 0.1–0.2 pH unit shift in 30–40 min. and 0.5–0.9 pH unit in 1½ hours. This was considered very satisfactory.

Since crystalline serum albumin was thromboplastic in the first phase reaction, it was not included in this study of pH in relation to antiprothrombic action.

Controls. With our particular reagents, the controls show a pH optimum surprisingly far into the alkaline region (*cf.* 16). Although the data of these and some other similar experiments define the optimum only within 1 pH unit, it is repeatedly demonstrated that the optimum is in the zone, pH = 8–9. There was a slight inhibition on increasing the alkalinity to pH = 10.0 and a much more striking inhibition in the acid region (pH = 5.0–6.0), the latter apparently associated with a heightened thrombin destruction.

Salmine. Two separate experiments, on different occasions, are included in table 9. In both cases brain thromboplastin was used as the

activator (with Ca) of the prothrombin. Comparison of the controls (a) and salmine (b) in experiment A shows that the antiprothrombic action of the salmine was exhibited, especially in the early phases, throughout the pH range 6.0–10.0. Experiments at pH 8.0 and 10.0 were also performed on the second occasion, but are not included in series B since the results were similar to the foregoing. The data of experiment B at pH = 6.0 and 5.0 give evidence of an *initial thromboplastic action* instead. In no. 5a this was followed by the usual inhibitory effect, but in no. 6a this did not develop within the time limit (1 hr.) of the tests.

TABLE 9

Effect of pH on activation of prothrombin, with and without salmine

Clotting-times (seconds), at 38°C., for 1 cc. fibrinogen + 0.5 cc. sod. barbital buffer (pH = 7.0) + 0.5 cc. thrombic mixtures (T). T-mixtures = 4 cc. prothrombin + 0.5 cc. 1:1000 soln. salmine sulfate (or distilled water) + 0.25 cc. dil. brain extract + 0.25 cc. N/10 CaCl₂, incubated at 10°C. for times indicated (minutes).

	pH	5 minutes	15 minutes	30 minutes	60 minutes
		sec.	sec.	sec.	sec.
A					
1a (Salmine)	10.0	220	110	60	20
1b (Control)	10.0	20	5	4	4½
2a (Salmine)	9.0	155	65	25	8
2b (Control)	9.0	6	4	4	4½
3a (Salmine)*	7.0	220	110	40	12
3b (Control)	7.0	35	16	10	5
4a (Salmine)*	6.0	275	295	315	400
4b (Control)*	6.0	133	130	87	65
B					
5a (Salmine)*	6.0	60	90	450	375
5b (Control)*	6.0	220	220	210	115
6a (Salmine)*	5.0	60	100	225	240
6b (Control)*	5.0	1140	1440	2040	1500

* Flocculation.

Heparin. Table 10 illustrates a similar study with heparin. Here again, the antiprothrombic action is manifest throughout the pH range (5.0–9.0). At no time was heparin thromboplastic. The sharp rise in anticoagulant effect noted in the last two readings at the acid values (pH = 5.0–6.0) was not progressive between 60 and 90 minutes and suggests an antithrombic rather than an antiprothrombic action. The controls show a similar, though much less marked, thrombin instability.

DISCUSSION. The present data, selected from a considerable number of similar experiments, re-affirm the stand we have previously taken (5), in support of the original view of Howell and Holt (10), viz., that heparin

is indeed an "antiprothrombin," in the general sense of being an agent which retards or prevents conversion of prothrombin to thrombin. In emphasizing the importance of the *type* of thromboplastic agent as determining the degree and duration of the antiprothrombic action of heparin, it was pointed out (11) that failure to realize this was responsible for a number of current denials of the antiprothrombic effect. We would add that it is not necessary to postulate an additional plasma factor (*cf.* 12) for the heparin inhibition of thrombin formation in the presence of tissue thromboplastin, provided that the action is looked for early enough and a sufficiently weak thromboplastin is used. The accompanying data stress these points, particularly the quantitative aspects of heparin-thromboplastin interrelationships. A strong tissue extract overcomes the heparin

TABLE 10

Effect of pH on activation of prothrombin, with and without heparin

T mixtures = 4 cc. prothrombin + 0.5 cc. heparin (1:2000) (or distilled water) + 0.25 cc. dilute brain extract + 0.25 cc. N/10 $CaCl_2$, held at respective pH values, and low temperature (10°C.) for times (minutes) indicated. Clotting-times (seconds), at 38°C., for 1.0 cc. fibrinogen + 0.5 cc. sod. barbital (1 per cent), buffered at pH = 7.0, + 0.5 cc. T.

	pH	5 MINUTES	15 MINUTES	30 MINUTES	60 MINUTES	90 MINUTES
		sec.	*sec.*	*sec.*	*sec.*	*sec.*
1a (Heparin)............	9.0	107	42	50	55	
1b (Control)............	9.0	12	10	10	11	
2a (Heparin)............	7.0	257	83	45	36	
2b (Control)............	7.0	42	17	12	11	
3a (Heparin)............	6.0	535	275	185	1020	960
3b (Control)............	6.0	90	50	70	57	47
4a (Heparin)............	5.0	700	600	470	1000	960
4b (Control)............	5.0	200	125	120	220	212

inhibition in all but the first minute or two of the prothrombin activation: a weak (brain) thromboplastin, like cephalin, is ineffective, or retarded over a period of an hour or more, if sufficient heparin is used.

The experimental data do not distinguish between two underlying possibilities, viz. 1, a direct action of heparin on prothrombin ("antiprothrombic" in a strictly literal sense), and 2, some process of "neutralization" of the thromboplastic factor(s) by heparin ("antithromboplastic" or "antithrombokinase" action in the sense of several previous workers). There is a definite dearth of evidence favoring any direct relationships.

SUMMARY

An experimental analysis has been made of the effects of serum albumin, salmine, and heparin on the isolated first and second phases of blood-clotting reactions. Acid-base influences have been investigated.

Crystalline serum albumin has a nonspecific thromboplastic action in the prothrombin → thrombin phase and a slight effect (usually antithrombic) in the thrombin + fibrinogen interaction. These phenomena are unimportant in relation to natural coagulation mechanisms. The purified albumin *lacks* the ability, consistently present in crude plasma "albumin" preparations, of producing a marked synergistic antithrombic action, in conjunction with heparin.

Salmine is antiprothrombic in the first phase and fibrinoplastic in the second. The inhibition preponderates in plasma. Both actions are antagonized by heparin.

Heparin is antiprothrombic in the first phase, to a degree and duration depending on quantitative relations to the thromboplastic factors. In the second phase, it has a minor immediate (nonprogressive) antithrombic effect, exaggerated (usually) by albumin and antagonized by salmine.

The significant anticoagulant effects in these studies are the *antiprothrombic* actions of heparin and salmine. There is some evidence that the minor immediate antithrombic actions of heparin and albumin (especially additively) favor the natural progressive thrombin inactivator (i.e., antithrombin, in the sense of serum "antithrombin") but the so-called proantithrombic (antithrombinogenic) factor of plasma and serum has not been considered a part of these studies.

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THE RATE OF RESPIRATORY ADJUSMENT TO POSTURAL CHANGE[1]

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Most of the errors in measurement of respiratory functions caused by physiologic factors tend to result in values that are too high. These errors may be caused by conditions such as an insufficient period of rest, too recent ingestion of food, breathing against a resistance, or discomfort from the face mask. In view of the discrepancies between the basal respiratory volumes found by us (1) and those found previously by many other investigators, it seemed desirable to ascertain the factors responsible. One of the obvious technical differences was the greater length of the rest period in our experiments as compared with the rest period in experiments of others. While it is generally recognized that subjects respire less air per unit of time after a period of rest, we have been unable to find any information in the literature as to the rate or the extent of this decline in respiratory volume. In order to assess the increment in respiratory volume caused by breathing carbon dioxide, these increments must be measured from a baseline, and the establishment of this baseline is impossible unless the length of the required rest period is known. Most experimenters have felt that practice affects the results since any subject going through an unfamiliar test is likely to have some emotional response. If this be true, one would expect that respiratory volumes would decrease with repeated testing and that the stabilized level might be reached sooner.

The data given in this report allow us to determine the length of the necessary rest period for establishing stable respiratory volumes in young adult males, the effect of training on the rate of stabilization of respiratory volumes and the individual variations in the rate at which respiratory volumes are stabilized.

EXPERIMENTAL. A group of 31 white, male college students (N. Y. A.) from 18 to 24 years of age served as subjects. Each subject was tested at a uniform time of day (either in the morning or in the afternoon) and had not eaten for one hour prior to each test. A Siebe-Gorman half-mask was securely tied over the subject's face while he was standing, and immediately after he had assumed the supine position the collection of the expired air was begun. He breathed outdoor air warmed to room temperature. Inspired and expired air were separated by a pair of egg-shell valves floating on mercury which required a pressure of only 1.5 mm. of water in order to open. The expired air was collected in a pair of recording spirometers of the Tissot type, each with a capacity of 9.19 liters (at 0°C., 760 mm. Hg). The apparatus was arranged so that time was recorded automatically at the end of expiration of each 9.19 liter sample of air. Continuous samples of expired air were measured over a period of 30 to 40 minutes. Respiratory volumes in liters per minute were computed from the time required to breathe out each 9.19 liter sample and were corrected to 0°C. and 760 mm. mercury. Each subject served for four experimental periods on different days.

TREATMENT OF DATA. Because of the individual differences in respiratory volumes, interpolated values were used in the calculation of average curves. Estimates were made of the respiratory volumes at minute intervals in each experiment from curves in which total elapsed time was plotted as the ordinate and successive 9.19 liter units of air were plotted as the abscissa. The respiratory volumes in liters were thus obtained for each minute from one to thirty, and were corrected for the size of the subject by dividing the recorded volumes by the surface area as estimated from the height and weight according to the DuBois formula. Zero time was taken at the moment the subject assumed the supine position. The respiratory volume of each of the 31 subjects appeared in every point in all four tests. Values were obtained for 30 minutes in the first three tests and for 20 minutes in the fourth test.

The results of the experiments are shown in table 1 and figure 1.

A rapid decrement in the respiratory volume occurred with the assumption of the supine position and an average of 20 minutes was required before this decrement became insignificant. Although a decrease was apparent between 20 and 30 minutes, it was not statistically significant.

A comparison of the four curves indicates that practice does not significantly alter the rate at which the respiratory volume becomes stabilized. The decrement must therefore be the result of rest plus the physiologic adjustment to postural change rather than to psychological factors related to unfamiliarity with the experimental technique. It should also be noted that the actual respiratory volumes do not decrease as more tests are done—an indication that with adequate preliminary rest periods extended training is not necessary.

Although no effects of practice were discernible in the average curves for the entire group, it seemed possible that some subjects would attain stable respiratory volumes more quickly in later experiments than on first

TABLE 1

Average respiratory volume for successive minutes after assuming supine posture

N = 31 adult males

MINUTES OF REST	EXPERIMENT I		EXPERIMENT II		EXPERIMENT III		EXPERIMENT IV	
	Mn.†	σ_{Mn}.†	Mn.†	σ_{Mn}.†	Mn.†	σ_{Mn}.†	Mn.†	σ_{Mn}.†
1	5.08	0.22	5.20	0.24	5.08	0.23	5.29	0.22
2	4.56	0.20	4.47	0.17	4.57	0.18	4.57	0.18
3	4.27	0.17	4.19	0.14	4.23	0.14	4.33	0.17
4	4.05	0.15	3.99	0.13	4.06	0.14	4.11	0.16
5	3.97	0.14	3.87	0.13	3.92	0.13	3.97	0.15
6	3.85	0.14	3.79	0.13	3.81	0.12	3.88	0.14
7	3.74	0.13	3.71	0.13	3.73	0.12	3.75	0.14
8	3.69	0.14	3.69	0.13	3.73	0.13	3.72	0.14
9	3.62	0.12	3.64	0.13	3.71	0.14	3.65	0.13
10	3.56	0.12	3.59	0.14	3.65	0.14	3.57	0.13
11	3.50	0.11	3.57	0.13	3.60	0.13	3.56	0.12
12	3.46	0.11	3.56	0.13	3.57	0.13	3.53	0.12
13	3.46	0.11	3.53	0.12	3.57	0.13	3.52	0.12
14	3.45	0.11	3.49	0.11	3.58	0.13	3.52	0.11
15	3.46	0.11	3.46	0.10	3.54	0.11	3.49	0.11
16	3.48	0.11	3.44	0.10	3.53	0.10	3.49	0.11
17	3.54	0.13	3.46	0.10	3.46	0.09	3.47	0.12
18	3.55	0.15	3.43	0.09	3.43	0.10	3.45	0.11
19	3.48	0.14	3.36	0.09	3.42	0.08	3.41	0.11
20	3.49	0.14	3.39	0.09	3.42	0.08	3.44	0.11
21	3.42	0.12	3.34	0.09	3.39	0.09	*	*
22	3.44	0.12	3.37	0.09	3.43	0.09		
23	3.41	0.13	3.38	0.09	3.47	0.10		
24	3.41	0.13	3.39	0.09	3.52	0.13		
25	3.39	0.12	3.34	0.09	3.51	0.12		
26	3.37	0.11	3.34	0.09	3.47	0.10		
27	3.33	0.11	3.36	0.09	3.42	0.09		
28	3.29	0.11	3.34	0.09	3.40	0.08		
29	3.31	0.11	3.34	0.10	3.43	0.09		
30	3.36	0.11	3.37	0.10	3.43	0.09		

† Liters per square meter per minute.

* Experiment IV terminated at 20 minutes.

trial. To test this possibility, the time required for each subject to stabilize his respiratory volume in each test was determined. The average of these estimations as determined independently by two observers was used to plot the frequency distributions as shown in figure 2. The results con-

firmed the findings for the average curves for all subjects; the mean time for the first test run was 20.6 ± 0.62 minutes, for the second run 19.25 ±
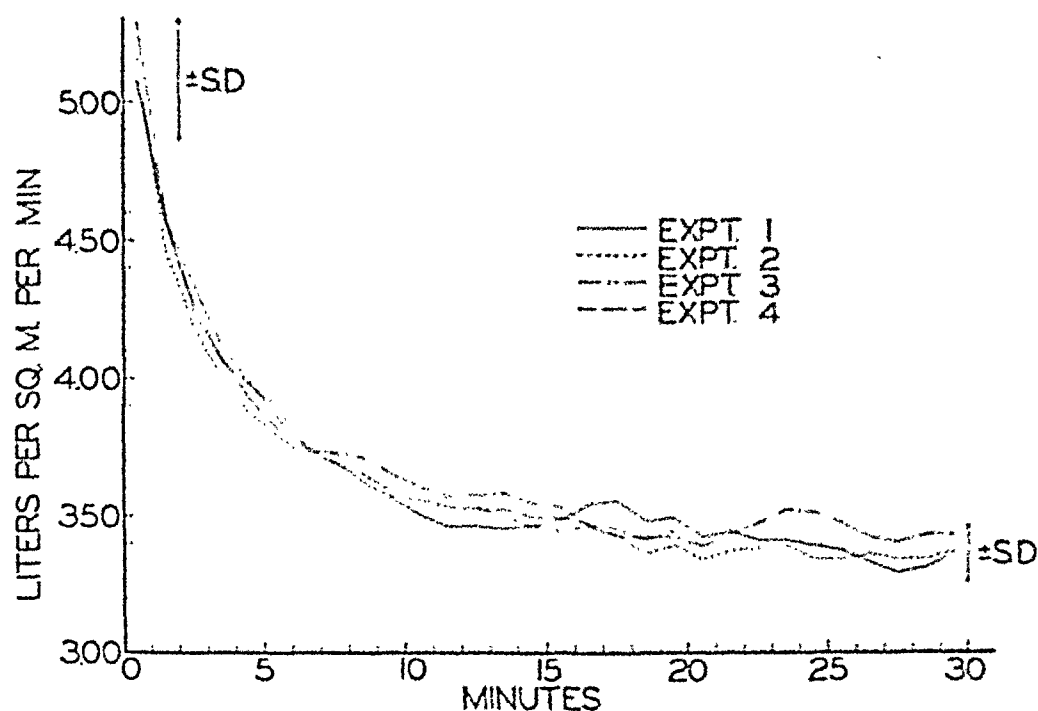
Fig. 1. Average decrement in respiratory volume after assuming supine posture. Average curves for 31 males—ages 18 to 21. Mn 23.16 years. Results from 4 experiments shows absence of learning effects. Length of arrow at beginning and end of curves shows ± 1 S. D. of the mean values. Zero time taken at moment of lying down. Mean values plotted at middle of temporal interval.

TABLE 2

Mean decrement in respiratory volume at 10-minute intervals after assuming supine position

N = 31

EXPERIMENT	MINUTE 1-10			MINUTE 10-20			MINUTE 20-30		
	Mn. l./sq.m./ min.	σ_{Mn}	C.R.†	Mn. l./sq.m./ min.	σ_{Mn}	C.R.†	Mn. l./sq.m./ min.	σ_{Mn}	C.R.†
I...........	1.52	0.18	8.2	0.07	0.10	0.7	0.13	0.05	2.4
II...........	1.61	0.19	8.6	0.20	0.07	2.5	0.02	0.03	0.7
III.........	1.44	0.17	8.3	0.22	0.07	3.1	0.01	0.04	0.3
IV..........	1.71	0.20	8.4	0.14	0.03	4.3	*	*	*

* Experiment IV terminated at 20 minutes.
† Critical ratio.

0.54 minutes and for the third run 19.01 ± 0.59 minutes. Inspection of the data from which figure 3 was derived showed that 6 of the 30 subjects required less time in runs 2 and 3 to stabilize their respiratory volumes

Fig. 2. Frequence distributions of the time required by individual subjects to attain stable values of respiratory volume.
—— test I; — – — test II; .... test III. Mean values shown by vertical lines. The same 31 subjects were used in each of the 3 tests.
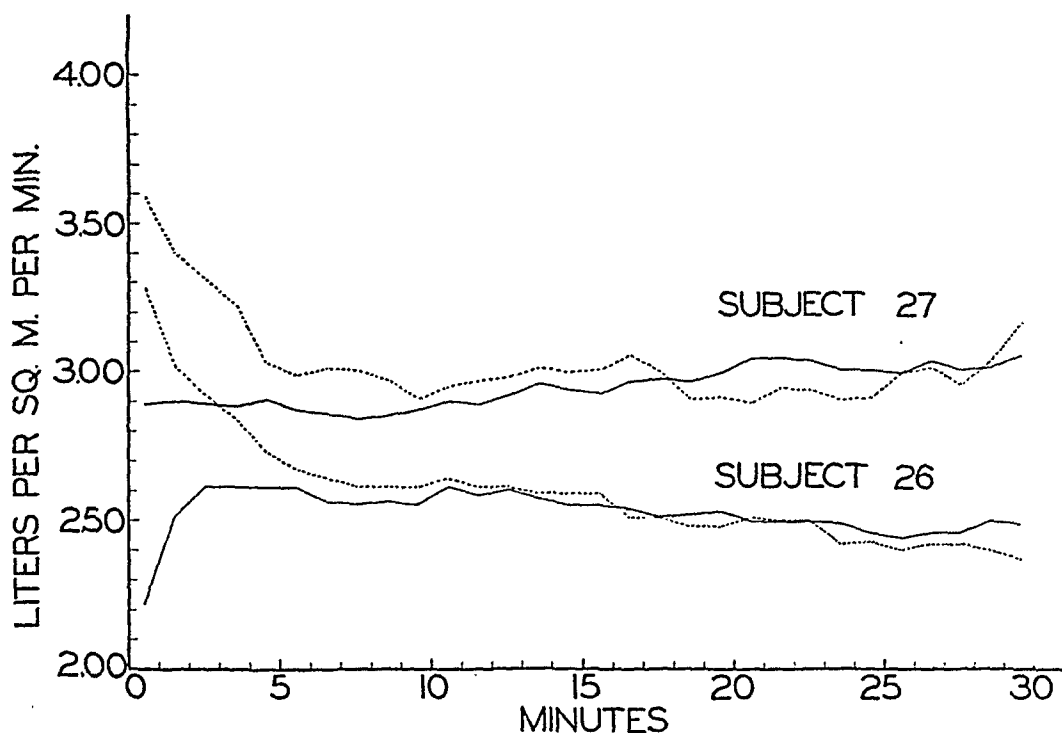
Fig. 3. Effect of postural change on decrement in respiratory volume.
—— Respiratory volume in individual subject after 30 minutes of rest in supine position. Zero time at 31 minutes after assuming supine posture.
.... Respiratory volume without previous rest. Zero time at moment of assuming supine posture.
Experiments on different days.

but that reversals of this effect in some of the other subjects were sufficient to establish the trend as shown in figures 1 and 2.

Table 2 shows that the decrement in respiratory volume is significant from the tenth to the twentieth minute but insignificant from the twentieth to the thirtieth minute.

In order to determine just what the effects of tying on the face mask would be, 3 subjects were put through the following experiment. Each subject rested for 30 minutes in the supine position. Then the Siebe-Gorman half-mask was tied to his face and the respiratory volumes were recorded as soon as the mask had been adjusted. The results (fig. 3) indicate that the respiratory adjustment was made during the 30-minute rest period and respiratory volume was not significantly increased by the application of the mask.

DISCUSSION. The results of this experiment show that a 20-minute rest period in the supine position suffices to stabilize the respiratory volume in most subjects while a 30-minute rest period is sufficient for practically all subjects. These periods of rest agree substantially with the findings of Benedict and Crofts (2) according to which a period of 30 minutes is long enough to stabilize oxygen consumption. However, our work also indicates that the minute respiratory volume under these standardized conditions is greater than the minute volumes under the fasting conditions which are routine in the measurement of basal oxygen consumption (2). Since previous observers have reported rest periods as long as 20 or 30 minutes, other factors must have caused the respiratory volumes to rise so much higher than they did in our experiments. These factors may have been: a greater resistance in the circuit, increased dead air space in the apparatus, and, in some cases, failure to reduce the volume to standard conditions of temperature and pressure.

SUMMARY

Continuous collections of expired air were made in a group of 31 young adult males immediately after they had assumed the supine posture, and were continued for 30 minutes. An average decrease in respiratory volume of approximately 40 per cent during the first 10 minutes and 10 per cent during the next 10 minutes was found. No significant decrease occurred between the twentieth and thirtieth minutes of rest. No evidence was obtained with successive testing that the rate of stabilization of the respiratory volume increased, although individual differences were noted. No significant alteration in respiration resulted from the application of a Siebe-Gorman half-mask.

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EFFECT OF OXYGEN TENSION OF INSPIRED AIR ON THE RESPIRATORY RESPONSE OF NORMAL SUBJECTS TO CARBON DIOXIDE[1]

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Opinions have differed widely concerning the influence of the alveolar oxygen tension on the respiratory response to carbon dioxide. Vernon (15) believed that high oxygen tensions decreased the respiratory response to carbon dioxide. Lindhard (9) and Hasselbalch and Lindhard (7) held that the respiratory center became more sensitive to carbon dioxide as the oxygen tension decreased. Campbell, Douglas, Haldane and Hobson (3) maintained that the alveolar oxygen tension could be varied within wide limits without sensibly affecting the respiratory response to carbon dioxide. The work of Eastman (5) and Selladurai and Wright (12) showed that the respiratory response to carbon dioxide is decreased in states of anoxemia.

The study of the relation of high oxygen tensions to the stimulating effects of carbon dioxide has received little attention. In fact, most of the investigations on carbon dioxide hyperpnea have been carried out without regard to the concentration of oxygen inhaled, except that in most instances anoxemia was avoided by using an "excess" of oxygen. To our knowledge Yamada (16) was the first to recognize that the inhalation of carbon dioxide-oxygen mixtures caused a greater respiratory increment than did the inhalation of carbon dioxide mixed with air. His experimental technique consisted of the measurements of alveolar carbon dioxide tensions. Davies, Brow and Binger (4) reported similar results but since "in some cases the percentage increase differed but little from the mean deviation," they were unwilling to lay any stress on their findings and felt that the matter could be decided definitely only "through statistical methods based upon a large amount of data collected under carefully

standardized conditions." In our own work an attempt has been made to clarify the effects of breathing several concentrations of carbon dioxide in pure oxygen and in 21 per cent oxygen by carefully controlled experiments with a larger number of subjects.

EXPERIMENTAL. Twenty-three male college students, ranging in age from 18 to 28 years, served as subjects. The number of subjects used for each concentration of carbon dioxide may be seen in table 2. Each subject was tested twice with a given carbon dioxide concentration in the presence of both 21 per cent oxygen and high oxygen. Each experiment consisted of 1, a period of 30 minutes during which the expired air was measured while the subject breathed outdoor air; 2, the test period during which the gas mixtures were breathed (this period was long enough for the respiratory volume to become stabilized), and 3, an after-period of 20

TABLE 1

Mean per cent increase in respiratory volume

	TEST I				TEST II				AVERAGE OF TEST I + II			
	Mn	σd	σMn	C.R.	Mn	σd	σMn	C.R.	Mn	σd	σMn	C.R.
100% O₂	13.7	15.2	3.6	3.8	12.4	8.0	2.1	5.9	13.3	11.4	2.7	5.0
1% CO₂ in air....	11.8	11.1	2.7	4.4	16.2	8.2	2.0	8.2	14.0	7.6	1.8	7.5
1% CO₂ in O₂.....	27.6	14.5	3.5	7.8	28.8	14.2	3.5	8.3	28.4	13.0	3.5	8.2
2% CO₂ in air....	30.9	15.2	4.4	7.1	37.2	16.1	4.6	8.0	34.2	13.2	3.8	8.9
2% CO₂ in O₂....	50.0	18.4	5.3	9.4	54.8	24.2	7.0	7.8	52.8	19.7	5.7	9.3
4% CO₂ in air....	97.1	35.8	8.7	11.2	105.4	41.5	10.4	10.2	101.3	36.5	9.4	10.7
4% CO₂ in O₂.....	122.2	47.1	11.4	10.7	124.6	57.5	14.4	8.7	121.1	45.4	11.7	10.3

minutes during which the subject again breathed outdoor air. Each subject was tested at a uniform time of day and had not eaten for one hour prior to each test. A Siebe-Gorman half-mask was adjusted to each subject and the expired air was measured over a period of 20 minutes while the subject was in the supine position in order to assure stable values of respiratory volume (13). The expired air was collected in a pair of recording spirometers of the Tissot type, each with a capacity of 9.19 liters (S.T.P.). These operated continuously in alternation and recorded electrically when each tank was filled. Egg shell valves (floating on mercury and opened by a pressure of 1.5 mm. of water) separated the inspired and expired air. After four preliminary trial runs, each on a different day, the experimental series was begun. Each subject was tested with only one mixture on any single day, but the tests with a similar mixture were repeated on a succeeding day.

In order to eliminate any effect of practice, the test series was counter-

balanced by having one-half the subjects breathe the carbon dioxide-air mixtures first and the other half the carbon dioxide-oxygen mixture first.

The gas mixtures were obtained in four 6000-liter high pressure tanks from which a pair of Tissot spirometers, each with a capacity of 60 liters,

TABLE 2

Effect of O_2 content on increment in respiratory volume resulting from increasing CO_2 in inspired air

Percentage increment in respiratory volume (based on average resting respiratory volume for each subject during fore-period)

SUBJECT NUMBER	1% CO_2			2% CO_2			4% CO_2		
	In 21% O_2	In 99% O_2	Diff.	In 21% O_2	In 98% O_2	Diff.	In 21% O_2	In 96% O_2	Diff.
1	12	61	49						
2	19	42	23						
3	12	16	4						
4	19	37	18						
5	20	23	3						
6	11	15	4	37	41	4			
7	8	30	22	34	49	15			
8	10	23	13	36	49	13	74	88	14
9	4	14	10	38	42	4	146	181	35
10	12	20	8	18	44	26	89	109	20
11	31	31	0	44	65	21	140	201	61
12	13	38	25	37	54	17	68	109	41
13	16	19	3	38	57	19			
14	21	35	14	22	71	49	108	202	94
15	9	23	14	24	42	18	80	90	10
16	12	32	20	41	74	33	129	163	34
17	13	24	11	42	45	3	107	118	11
18							86	113	27
19							97	106	9
20							63	99	36
21							90	96	6
22							93	120	27
23							109	132	23
Mean....	14.2	28.4	14.2	34.3	52.8	18.5	98.6	128.5	29.9
$\sigma_{Mn. diff}$...............			3.0			4.1			6.3
C.R......................			4.7			4.5			4.7

was filled. The use of these large Tissot spirometers allowed the gas mixtures to come to the same temperature and pressure as the air in the room before being respired. One of the large spirometers was filled from the storage tanks while the subject breathed the mixture from the other. The intake valves were arranged so that the mixtures could be connected

to the inspiratory circuit without the knowledge of the subject. The carbon dioxide content of the gas mixtures from the high pressure tanks and from the large Tissot spirometers was measured before each separate test and was found to vary less than ±0.01 per cent from the purported value.
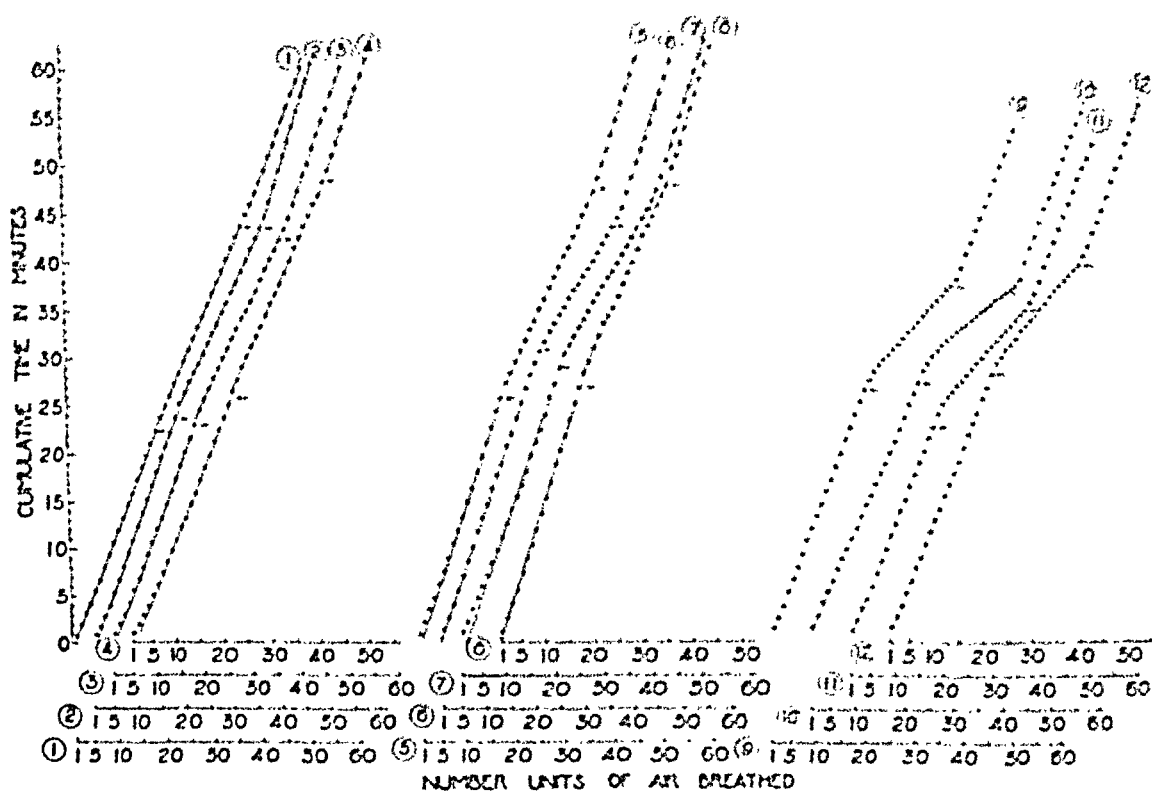
Fig. 1. Effect of alterations in O_2 tension on increase in respiratory volume produced by low concentrations of CO_2 in inspired air in normal male. Ordinate-cumulative time in minutes required to expire equal units of air (9.1 l.). CO_2-O_2 mixtures administered over intervals between arrows. All 12 experiments on single subject age 23. Height 176.6. Weight 77.4. Curves *1* and *4* are duplicate experiments in which a mixture of 1 per cent CO_2, 21 per cent O_2, and 78 per cent N_2 was administered. In curves *2* and *3* a mixture of 1 per cent CO_2, 99 per cent O_2 was administered. In curves *5* and *8* a mixture of 2 per cent CO_2, 21 per cent O_2 and 77 per cent N_2 was administered. In curves *6* and *7* a mixture of 2 per cent CO_2 and 98 per cent O_2 was administered. In curves *9* and *12* a mixture of 4 per cent CO_2, 21 per cent O_2 and 75 per cent N_2 was administered. In curves *10* and *11* a mixture of 4 per cent CO_2 and 96 per cent O_2 was administered. Experiments were run in the temporal order as numbered.

ANALYSIS OF DATA AND RESULTS. Respiratory volumes were computed by dividing the volume of expired air in each 9.19 liter tank (corrected to 0°C. and 760 mm. pressure) by the time required to fill each tank, and were recorded as volumes in liters per minute or as volumes in liters per square

meter per minute. In all the experiments the resting level was determined from an average of at least 10 observations following the 20-minute rest period. In order to decide which points should be included in the determination of the resting level, graphs were constructed (see fig. 1) in which cumulative time was plotted as the ordinate and the number of tanks filled with expired air was plotted as the abscissa.[2] Straight lines were fitted by free-hand methods to the experimental points so plotted. As can be seen in figure 1, these points form a straight line when the respiratory volume is constant. Increases in respiratory volume result in a decrease in the slope of the line. Average values were computed using only points after a linear trend had been established. The resting level was used as the value from which all deviations caused by breathing the carbon dioxide mixtures were computed. Table 1 is a summary of average results from all the subjects calculated in per cent, together with the critical ratios (computed as ratio of the mean difference between resting respiratory volume and stimulated volume divided by the standard error of the difference). The increments in table 1 are based on the averages of two tests for each subject, with the baseline calculated on the fore-period.

Table 1 indicates that carbon dioxide mixed with 21 per cent oxygen causes a significant increase in the respiratory volume in all concentrations used. One per cent carbon dioxide causes a mean increase of approximately 14 per cent, while 2 per cent carbon dioxide causes an increase of about 34 per cent. We found, as have others, that 4 per cent carbon dioxide doubles the respiratory volume. On the other hand, when the same concentrations of carbon dioxide are mixed with pure oxygen, the increments are much greater. Thus, 1 per cent carbon dioxide in oxygen increases the respiratory volume almost as much as does 2 per cent carbon dioxide in air.

Since each subject breathed a given percentage of carbon dioxide in 21 per cent oxygen and also the same percentage of carbon dioxide mixed with pure oxygen, the significance of the difference in respiratory response to carbon dioxide under the two conditions was tested by the method of differences, as summarized in table 2. This table shows that the response to all concentrations of carbon dioxide mixed with pure oxygen is significantly greater than to the same concentrations of carbon dioxide mixed with 21 per cent oxygen, since the critical ratios are 4.7, 4.5 and 4.7 for 1, 2 and 4 per cent carbon dioxide.

DISCUSSION. A possible explanation of our results is that in the presence of a high oxygen tension in the respiratory center the sensitivity of these cells to the normal stimulus of increase in hydrogen ion concentration may

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[2] This method of plotting was utilized because fewer calculations were required and because this method tends to minimize the effects of chance fluctuations.

be enhanced so that respiration is increased without a measurable rise in the hydrogen ion concentration or in carbon dioxide of the center. It is true that if 13.3 per cent (the average increment in respiratory volume produced by breathing 100 per cent oxygen) is deducted from the values obtained when carbon dioxide mixed with pure oxygen is inspired (table 2), the discrepancy is 0.9 per cent $(14.2 - (28.4 - 13.3))$ when breathing 1 per cent carbon dioxide; 5.2 per cent $(31.3 - (52.8 - 13.3))$ when breathing 2 per cent carbon dioxide; and 16.6 per cent $(98.6 - (128.5 - 13.3))$ when breathing 4 per cent carbon dioxide. If these increasing differences were statistically significant, it would prove that the respiratory center is more sensitive to a given concentration of carbon dioxide when the oxygen tension is increased. However, the differences, although suggestive, are not statistically significant.

Since it has been shown previously that a significant rise in respiratory volume results from breathing 100 per cent oxygen (14) we are forced to conclude that the results observed represent only an additive effect of increased O_2 tension and increased CO_2 tension. Since the differential effect does not disappear with breathing 4 per cent CO_2 we doubt whether it can be attributed to a reduction in blood flow in the brain which Lennox and Gibbs (8) and other investigators (10, 11) have found in humans and mammals breathing pure oxygen.

The rise in CO_2 tension of the tissues of animals breathing pure oxygen reported by Campbell (2), as well as the rise in CO_2 tension of both arterial and venous blood in animals breathing pure O_2 at 4 atmospheres' pressure reported by Behnke, Shaw et al. (1), lend support to the thesis originally proposed by Gesell (6) that in the presence of high oxygen tension the transport of carbon dioxide from the tissues might be interfered with because the amount of reduced hemoglobin available to transport a given amount of CO_2 would be reduced as a result of the increased amount of oxygen carried in physical solutions.

Practically, the conclusion to be drawn from our work is that a given concentration of carbon dioxide should be mixed with oxygen rather than with air to produce a maximal degree of hyperpnea. Conversely, (as concluded from the work of others), in states of anoxemia oxygen should be used in high concentration in order to enhance the respiratory response to either the carbon dioxide present or to the carbon dioxide that may be added.

SUMMARY. A group of 31 adult males has been studied. Each subject rested for 30 minutes in order to stabilize the respiratory volumes and then breathed (through a Siebe-Gorman half-mask) a mixture of 1 per cent carbon dioxide and 21 per cent oxygen for a period of 8 to 15 minutes, after which time the respiratory volume was again stable but at a higher level. This experiment was repeated with 1 per cent carbon dioxide and 99 per

cent oxygen. In all the experiments the average increment in the respiratory volume was greater when the subject breathed 1 per cent carbon dioxide in 99 per cent oxygen than when he breathed 1 per cent carbon dioxide in 21 per cent oxygen. Similar results were obtained when 2 and 4 per cent carbon dioxide were mixed with oxygen. In some subjects the difference in respiratory response was great enough to be of clinical importance and should be considered when carbon dioxide is administered for therapeutic purposes.

CONCLUSIONS

1. Data are shown which give the average effect of 1, 2 and 4 per cent carbon dioxide on the respiration of normal adult males.

2. Normal subjects respond with a greater increment in respiratory volume to carbon dioxide (in concentrations of 1, 2 and 4 per cent) mixed with pure oxygen than to the same concentrations of carbon dioxide mixed with air.

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PRESSOR RESPONSES FOLLOWING SHORT, COMPLETE RENAL ISCHEMIA: CHARACTERISTICS, MECHANISM, SPECIFICITY FOR KIDNEY

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That an elevation of blood pressure follows the restoration of renal circulation after periods of complete arrest has been reported by Katzenstein (1905), Dicker (1937, 1938), and Taquini (1938, 1939, 1940). In a preliminary publication Collins and Hamilton (1940) confirmed the results of Taquini, consistently obtaining elevations of blood pressure after 5½ to 6½ hours of complete, bilateral renal ischemia. At the same time Friedberg, Landowne, and Rodbard (1940) reported similar experiments, but obtained definite elevations of blood pressure in only 8 of 19 experiments. Later Prinzmetal, Lewis, and Leo (1940) uniformly observed pressor responses upon reëstablishing the circulation through one kidney after 4 to 6 hours of complete bilateral ischemia.

While extensive studies of chronic partial ischemia have been made, no comparable investigations exist for temporary complete ischemia. The latter may prove a valuable tool in the investigation of renal hypertension, and warrants a more detailed study. We have, therefore, considered the following phases of this problem: short periods of complete ischemia, unilateral ischemia, characteristics of pressor response, rôle of nervous mechanisms, effects of previous adrenalectomy and splenectomy, and ischemia of other organs.

METHODS. Dogs were anesthetized with chloralose except in 2 experiments where sodium pentobarbital was used. Through a mid-line abdominal incision all collateral circulation was destroyed by separating the kidneys from surrounding peritoneum and fat and by tearing visible blood vessels on the ureters. The artery and vein of each kidney were occluded by a special screw clamp provided with a sleeve extension, which passed through an abdominal stab wound. After closure of the abdomen, the clamps could be manipulated by the sleeve extensions without disturbance to the animal. Renal circulation was always examined at the end of the experiment, and was found to be reëstablished in all cases. Mean arterial blood pressure was recorded by a mercury manometer, usually from the femoral artery.

RESULTS. *Complete bilateral ischemia lasting 6 to 7 hours.* When the clamps, occluding the vessels of the 2 kidneys, were opened simultaneously after periods of complete ischemia similar to those used by Taquini (1938, 1939, 1940), elevations of blood pressure were consistently obtained (table 1).

TABLE 1

Periods of 6 to 7 hours of complete, bilateral renal ischemia

	EXPERI-MENT 1	EXPERI-MENT 2	EXPERI-MENT 3	EXPERI-MENT 4*	EXPERI-MENT 5*
Length of ischemia..................hrs.	$6\frac{1}{2}$	$6\frac{1}{2}$	7	7	$6\frac{1}{6}$
Original level of blood pressure...mm. Hg	132	146	112	123	116
Maximum elevation of blood pressure mm. Hg	48	20	50	34	26
Elevation at end of experiment....mm. Hg	38	18	38	32	24
Time between unclamping and end of experiment.........................min.	24	27	16	21	15

* The animal was anesthetized with sodium pentobarbital.

TABLE 2

Shorter periods of complete, bilateral renal ischemia

	EXPERIMENT 6	EXPERIMENT 7	EXPERIMENT 8	EXPERIMENT 9	EXPERIMENT 10	EXPERIMENT 11	EXPERIMENT 12	EXPERIMENT 13*	EXPERIMENT 14
Length of ischemia............hrs.	3	$3\frac{1}{4}$	$3\frac{1}{6}$	$2\frac{1}{4}$	2	1	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$
Original level of blood pressure mm. Hg	140	152	109	84	118	114	120	58	127
Maximum elevation of blood pressure mm. Hg	24	16	44	34	28	32	22	33	30
Elevation at end of experiment mm. Hg	24	6	44	31	28	32	14	24	30
Time between unclamping and end of experiment.................min.	49	18	36	25	19	46	24	65	9

* Blood pressure was recorded from the carotid artery.

Shorter periods of complete bilateral ischemia. The next series of experiments involved periods of ischemia shorter than those studied by other investigators. Pressor responses were uniformly obtained even with periods as brief as ½ hour (table 2, figs. 1 and 2 B). The length of the period of ischemia exerted only a slight effect on the magnitude of the response (tables 1 and 2). As the time was shortened, there was less damage of the kidney as evidenced by more normal gross appearance after

restoration of circulation. These organs, therefore, resemble the partially ischemic kidney (which may show no demonstrable damage) more closely than do those studied by previous investigators, using longer periods.

Characteristics of the pressor response. In many cases the pressor response consisted solely of a gradual elevation of blood pressure, beginning as a rule almost immediately after opening the clamps. In some experiments, however, this gradual rise was preceded by preliminary fluctuations.
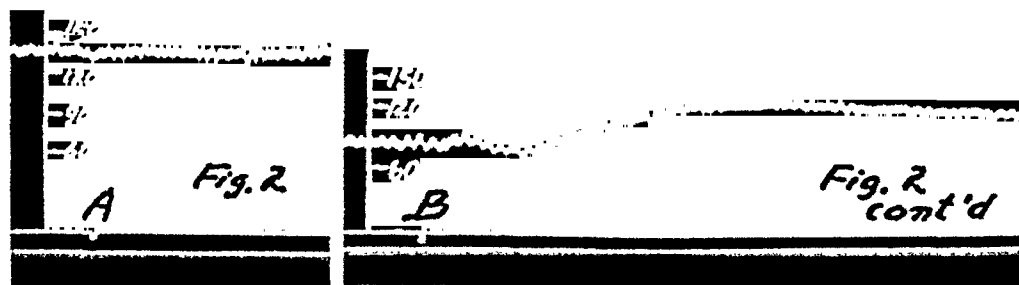
Fig. 1. Dog. 8. Restoration of circulation after 3½ hours of complete, bilateral renal ischemia. The clamps were opened at the signal.

Fig. 2. Control experiment. The clamps were applied to both pairs of renal vessels, previously ligated with linen thread, together with the intact, accompanying nerve plexuses. At A the clamps were opened, 1½ hours after their closure. The clamps were then retightened, and the linen ligatures were removed. At B, 45 minutes after A, the clamps were opened again.

In the figures the first line is the femoral blood pressure, the second is the base and signal line, and the third is a time line of 4 second intervals.

In most cases they consisted of a rapid rise followed by a fall to a level usually, but not always, below the original pressure (fig. 1). A nervous mechanism is not involved as experiments to be described later showed that these preliminary fluctuations were not produced by control manipulations or sham opening of the clamps and were present after denervation of the kidney and destruction of the cord combined with vagotomy. Furthermore, they are not dependent upon the presence of the adrenals or spleen, since they occurred after excision of these organs.

The blood pressure reached its maximum height 2 to 9 minutes after unclamping. The longer delays were usually associated with marked preliminary variations. The pressor response was prolonged (tables 1 and 2). In some of the subsequent experiments the blood pressure was recorded for an hour or more after the release of the clamps, and it did not fall to its original level in this time.

When all the experiments were analyzed, the magnitude of the response was found to be independent of the initial value of the blood pressure. In some of the animals, in which much of the spinal cord was destroyed, poor and atypical rises occurred when the blood pressure was low and continuously falling.

Complete unilateral ischemia. In 7 experiments the pressor responses from one kidney were studied; the second kidney was intact in 4 cases, and clamped in the other 3. The periods of ischemia varied from $1\frac{1}{2}$ to $8\frac{1}{4}$ hours, but most of them were less than $3\frac{1}{2}$ hours. In all instances typical rises of blood pressure (16 to 34 mm. Hg) occurred when the circulation through the single kidney was restored. Numerical values for elevations of blood pressure always refer to the maximum height of the prolonged rise, and not to the preliminary fluctuations.

Absence of a nervous mechanism. Experiments were next performed to determine what rôle, if any, nervous factors play in the response.

1. *Manipulation of the clamps.* In many of the experiments the clamps were either rapidly closed and opened or purposely pulled and twisted to a degree calculated to produce an amount of stimulation equal to that involved in opening the clamps; no significant changes of blood pressure resulted.

2. *Sham opening of the clamps.* Three control experiments, similar to those of Taquini (1938, 1939, 1940), were performed. After collateral circulation had been destroyed, clamps were applied to both pairs of renal vessels, previously ligated with linen thread, together with the intact accompanying nerves. When the clamps were opened $1\frac{1}{2}$ to 2 hours later, no change of blood pressure occurred (fig. 2 A). The experiments were continued by retightening the clamps and removing the ligatures. When the clamps were now opened, the usual rise of blood pressure occurred (fig. 2 B).

3. *Complete ischemia of denervated kidneys.* In 4 dogs, after separation of the kidneys from surrounding tissues, the ureters and the entire lengths of the renal vessels were mechanically stripped of nerves and generously painted with phenol solution. Following denervation the kidneys were subjected to about 2 hours of complete ischemia. Pressor responses occurred upon restoration of circulation (24, 30, 44, and 66 mm. Hg).

4. *Complete ischemia after cord destruction and vagotomy.* In 4 dogs most or all of the cord below the cervical segments was destroyed by a

heavy hooked wire, inserted into the spinal canal through a laminectomy. The extent of complete destruction, ascertained by opening the spinal canal at the end of the experiment, was as follows: dog 1—all below the first thoracic roots, dog 2—portion between the first thoracic and fifth lumbar roots, dog 3—all below the fifth cervical roots, dog 4—all below the sixth cervical roots. After the kidneys were rendered ischemic, the vagi were sectioned. The duration of the initial ischemia varied from 1 to 1½ hours. In 3 of the animals the kidneys were subjected to a second period of ischemia (⅔ to 1½ hrs.). In 4 of the 7 unclampings prolonged elevations of blood pressure of 14 to 30 mm. Hg resulted, preceded with one exception by unusually prominent preliminary variations. In the other 3 unclampings, where the blood pressure was low and continuously falling, the pressor responses were small or poorly maintained.

The preceding series of experiments indicate that the pressor response is of non-nervous origin; consequently a humoral mechanism must be involved. This conclusion coincides with that drawn by Taquini principally from different types of experiments. In addition, the last group of experiments indicates that the renal pressor material does not act exclusively, if at all, through the central nervous system since the thoracolumbar cord, from which the sympathetic vasoconstrictor fibers arise, was largely or entirely destroyed.

Complete ischemia of structures other than the kidney. It is important to determine whether the phenomenon is a special property of renal tissue. Other structures were, therefore, studied.

1. *Leg.* In 3 dogs the hind leg was completely isolated, except for the blood vessels, at a point just above the knee joint. The vessels were then occluded for 1½ to 2 hours, during which time the leg was kept at about body temperature. When the clamp was opened, there was no significant change in blood pressure in any of the 3 animals. Circulation was found to be intact at the conclusion of the experiments.

2. *Liver.* In 3 dogs the circulation through the narrow pedicle-like root of the left division of the central lobe of the liver was completely obstructed for about 2 hours. When the clamp was released, a fall of blood pressure occurred in one animal and no change in 2. At the end of the experiments examination showed that circulation had been restored. The weight of the hepatic tissue subjected to ischemia varied from 63 to 119 grams.

Thus, as far as leg or liver is concerned, the pressor phenomenon is specific for the kidney.

Complete, bilateral renal ischemia after adrenalectomy. After recent bilateral adrenalectomy characteristic pressor responses were obtained as follows: dog 1, 18 mm. Hg (2½ hrs.); dog 2, 36 mm. Hg (2 hrs.), 18 mm. Hg (additional 2 hrs.); dog 3, 37 mm. Hg (3⅙ hrs.).

Complete renal ischemia after splenectomy. In 3 recently splenectomized dogs, periods of complete bilateral ischemia of $1\frac{2}{3}$, $1\frac{1}{10}$, and $2\frac{5}{6}$ hours gave elevations of blood pressure of 32, 36, and 46 mm. Hg respectively.

DISCUSSION. The conclusion that the elevation of blood pressure following restoration of circulation through the completely ischemic kidney is due to the liberation of pressor material from this organ is supported by other observations in the literature. These observations will be briefly summarized.

Taquini (1938, 1939, 1940) obtained pressor responses by transplantation of completely ischemic kidneys (about 6 hrs.) or by injection of their venous blood. Non-ischemic kidneys gave negative results. Using the Läwen-Trendelenburg preparation he found vasoconstrictor properties in venous plasma from completely ischemic kidneys, but not from normal kidneys or completely ischemic spleens. Mason and Rozzell (1939), however, found the Läwen-Trendelenburg preparation unsatisfactory for the study of vascular effects of dog sera.

Pressor responses have been obtained from perfusates of kidneys rendered completely ischemic for 24 hours (Dicker, 1937, 1938) and 4 to 6 hours (Prinzmetal, Lewis and Leo, 1940). Williams and Grossman (1938) found pressor substances in renal perfusates. Although the kidneys were not purposely made ischemic, a period of a few minutes to 2 hours or more elapsed before the perfusions were performed. These authors also found 2 active substances in such perfusates, one resembling epinephrine, the other renin; this claim may bear a relationship to the preliminary variations obtained in our experiments. All the above investigators failed to find pressor activity in perfusates of completely ischemic organs other than the kidney. This finding agrees with our results on leg and liver.

While it appears likely that the pressor material involved in persistent hypertension from partial renal ischemia is closely related to the agent involved in complete ischemia, the relationship has by no means been clarified. However, it is significant that Loesch (1933) was able to produce moderate persistent hypertension by repeated brief occlusions of the renal vessels and ureters.

SUMMARY AND CONCLUSIONS

Taquini's findings are confirmed; intervals of complete, bilateral renal ischemia of 6 to 7 hours invariably result in elevations of blood pressure when circulation is restored.

Briefer intervals of complete renal ischemia, even those as short as $\frac{1}{2}$ hour, consistently give pressor effects. The magnitude of the responses is only slightly less than that from longer periods.

The characteristics of the response are as follows: the blood pressure

rises gradually, reaching a maximum 2 to 9 minutes after the release of the occluding clamps; the elevation is prolonged; in some cases the gradual rise is preceded by preliminary changes, consisting usually of a relatively rapid rise followed by a fall; these preliminary variations are not due to stimulation incident to opening the clamps; the gradual prolonged response is independent of the height of the initial blood pressure.

Occlusion and subsequent restoration of the blood supply of one kidney, either with the other intact or clamped, is followed by a prolonged elevation of blood pressure.

A nervous mechanism is not involved in these responses. This conclusion is based on 4 groups of experiments: 1, manipulation of the clamps; 2, opening of the clamps with the vessels of the kidney ligated and the accompanying nerve plexuses intact; 3, complete ischemia with the kidneys and their vessels denervated; 4, complete ischemia with the thoracolumbar cord destroyed and the vagi cut. The responses must, therefore, be due to pressor material from the ischemic kidney. This material does not exert its pressor action solely, if at all, through the central nervous system.

If the circulation to the leg or to a portion of the liver is arrested for about 2 hours, no elevation of blood pressure occurs when the circulation is restored.

Neither recent adrenalectomy nor recent splenectomy prevents an elevation of blood pressure following restoration of renal circulation after complete bilateral ischemia.

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INTRALUMEN PRESSURES OF THE DIGESTIVE TRACT, ESPECIALLY THE PYLORIC REGION[1]

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The literature on the digestive tract contains numerous references to the intralumen pressures of the gut. The limitations of the methods commonly employed in such investigations should be appreciated, for in general they are physically inadequate to supply quantitative values. The fluid or semifluid gastro-intestinal contents will tend to move from a region of higher to a region of lower intralumen pressure; thus observations, usually roentgenographic, of direction and rate of translocation of contents afford a rough suggestion of pressures.

Records obtained by the balloon-water manometer technic have frequently been interpreted in terms of intralumen pressure, but the objections to this system have been so universally unappreciated that a critical analysis is desirable. Certain objections are referable to the water manometer. Since it has a high volume-pressure coefficient it cannot yield quantitative values. This was illustrated as follows: With a 3 x 10 cm. balloon in a pressure chamber, but closed off from its water manometer, introduction of 5.5 cc. of air into the chamber produced a pressure of 10 cm. of water. On restoring connection between the balloon and water manometer, the pressure fell to 5.2 cm. An additional 5 cc. of air in the chamber was required to restore the pressure to 10 cm. Also, unless the balloon contains a large volume, displacement of its entire contents may be insufficient to record the maximum pressure applied to the balloon. However, a balloon of large volume constitutes an undesirable foreign body and it records from a large region, not from a point.

The pressure, P, developed at any instant within a cavity whose size is changing is related to the time rate of volume change $\left(\dfrac{dv}{dt}\right)$ and to the resistance offered to the escape of contents, R, as shown by the formula $P = \dfrac{dv}{dt}R$. A pressure device having a large volume-pressure ratio pro-

vides an abnormal channel for escape of material from the cavity in which pressure is to be measured. By reducing R, an error in pressure is introduced which bears no constant relation to the naturally occurring pressure. This abnormal channel for escape of material alters both physically and physiologically the efficiency of the cavity as a pressure creating mechanism. Substitution of a bromoform manometer, a mercury man-
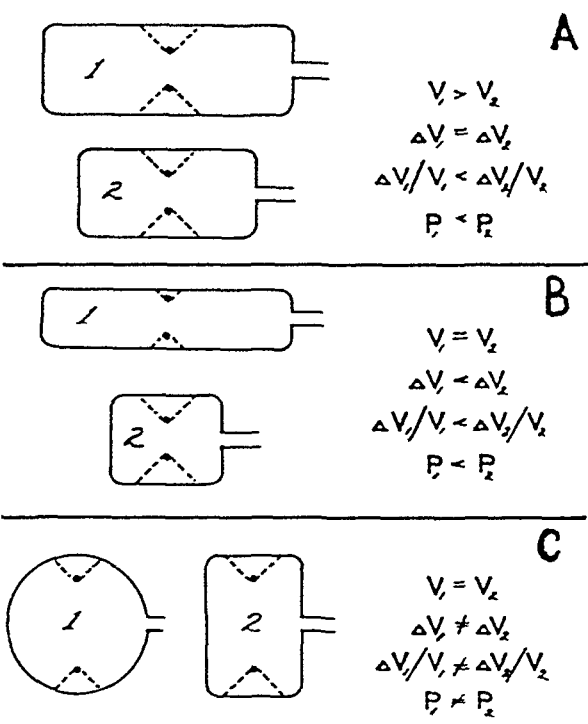
Fig. 1. The pressure produced by a ring slipped over a balloon will vary according to the volume, size and shape of the balloon. With each pair of balloons the constricting rings have the same diameter. A. The balloons have the same shape and diameter but not the same volume. B. The balloons have the same shape and volume but not the same diameter. C. The balloons have the same volume and diameter but not the same shape.
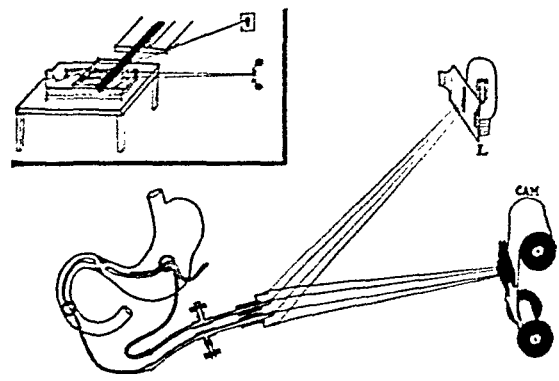
Fig. 2. Method of optical registration of pressure in the pyloric antrum and duodenal bulb.

ometer, a recording tambour, or an optical manometer progressively decreases the magnitude of the error due to volume change.

The balloon has certain inherent objections when used for the quantitative measurement of pressure. When the pressure is applied by a fluid medium, uniformly to the entire balloon surface, as in a pressure chamber or the "encased balloon" method (discussed subsequently), these objec-

tions are minimal. When the balloon is distorted into a dumb-bell shape by a constricting ring, such as by the direct contact of a peristaltic contraction of the gut, pressures will be dependent to an unpredictable extent on the volume, size and shape of the balloon (fig. 1). Also, if the constricting ring causes a decrease in balloon volume, artificial pressures, not existing in the gut lumen, may develop in the balloon. These pressures will be inversely related to the ease with which the rubber stretches. We have recorded pressures from a small balloon (having an effective volume of 0.3 cc.) in the gut 2 to 3 times as great as from a physically accurate recording device fastened adjacent to it.

TECHNIC. Although the principles of accurate pressure registration have been well established by Frank (1), they have not previously been applied to the determination of gastro-intestinal pressures. We have applied these principles in constructing an optical manometer which consists of a brass tube with 1.5 mm. bore connected to the portion of the gut to be studied by lead and rubber tubing having an internal diameter of 1 mm. The total length was about 45 cm. The recording end of the manometer was closed with a rubber membrane supporting a small mirror chip which received light from a slit lamp and reflected the beam to a photokymograph 2.5 meters from the mirror (fig. 2).

The maximum rate of motility in the pyloric sphincter region may be taken as 30 contractions per minute and the frequency of the fastest significant component as 15 times this or 450 per minute (7.5 per sec.). This manometer is adequate to record such pressure changes without distortion since it has a higher vibration frequency; 75 per second when recording from air, 20 per second when recording from water or gastric juice and 15 per second from cornmeal mush. The vibration frequency of an ordinary water manometer recording from air is 1 to 2 per second. The coefficient of damping of our manometer is greater than 0.9 (i.e., the oscillations are practically critically damped) so it is capable of faithfully recording sinusoidal pressure waves of nearly 15 per second from cornmeal mush. Application of Fourier's method of harmonic analysis to the curve shows that the error in the registration of maximal values is less than 1.3 per cent. The sensitivity of the manometer is altered at times to meet special requirements by varying the thickness and tension of the rubber membrane but usually a deflection on the photokymograph of about 2 cm. is obtained for 10 cm. of water pressure and the volume change in the apparatus is 0.1 cc. for 50 cm. of water pressure.

Open tube method. The "open tube method" was employed in most of our studies. In this method, the rubber tube in the gut terminates in a hollow cylindrical tip made of cellulose acetate which is firm enough to retain its shape, but soft enough to be practically non-irritating to adjacent tissue. The overall length of the tip is 2 cm., the free end has an internal
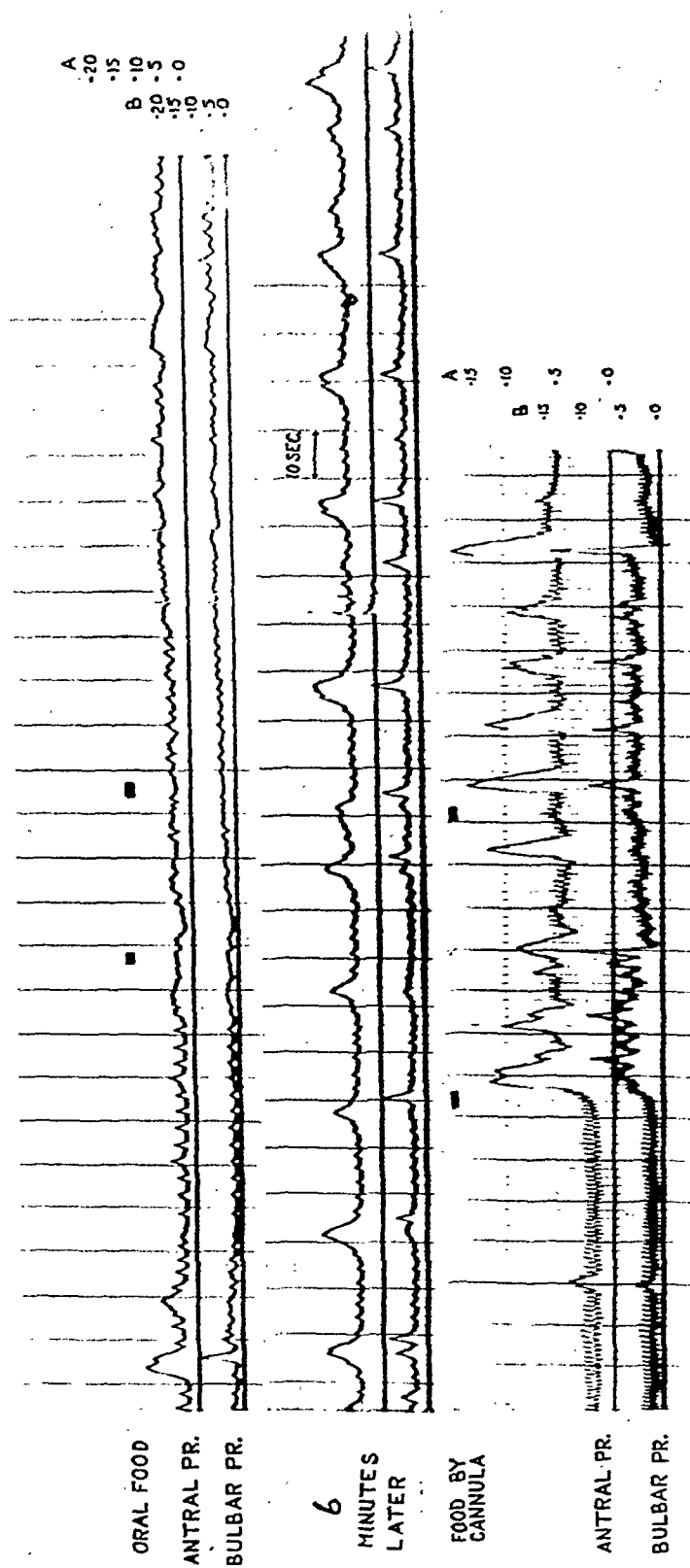
Fig. 3. Effect on antral and bulbar pressures of feeding by mouth (2 upper records) or by cannula (lower record). Interval of feeding indicated by the heavy horizontal marks.

diameter of 4 mm. and the end attached to the tubing is turned down to 1 mm. In determining the pressures within the pyloric antrum and duodenal bulb, two open cellulose acetate tips are drawn into the spaces to lie 3 mm. proximal and distal to the sphincter (fig. 2). In normal trained dogs provided with permanent metal cannulae by the technic of Meschan and Quigley (2), several hundred experiments have been performed. At the onset of the experiment food has been withheld for 24 hours. The animals are placed in the dorsal position in a comfortable hammock where they remain without restraining bands. When the recording tubes are connected to optical manometers as described, the intralumen pressure occurring at the mouth of each plastic tip is measured.

The records are not affected by pressure of the gut wall on the tube, and the volume change with pressure is not significant. The small tube in the gut has essentially no distending effect on the gut wall and the effect of the recording tip as a foreign body appears to be minimal.

Encased balloon method. We also recorded the intralumen pressure with a balloon 1 cm. long and 0.5 cm. diameter and containing when in use 0.3 cc. air. This is attached by a rubber tube to an optical manometer. This balloon is encased in a metal capsule having numerous perforations. When records are made simultaneously from the encased balloon and from an open tube lying adjacent to it, the phasic pressure changes are es entially identical. In general, results obtained from the open tube method and the encased balloon are identical except that the open tube records from the *point* at the mouth of the tube in the gut, while the encased balloon records from the larger space or *region* of the gut in which it lies. With the encased balloon, changes in temperature or position of the tubes may create false pressures (not true with the open tube method) and basal pressures are recorded less accurately. Furthermore, the encased balloon is a more objectionable foreign body than the open-tube. Passage of gastric contents into the encased balloon is impossible but such material may enter the open tube (it enters the plastic tip about 6 mm. for a pressure of 50 cm. water and a pressure greater than 150 cm. is necessary to force material into the narrow bore end). Possible obstruction of the open tube from this source is minimized by routinely blowing 1 cc. of air through the tube at intervals and always when the changes characteristic of plugging appear.

RESULTS AND INTERPRETATIONS. *The empty antrum and bulb form multiple transitory closed cavities.* It was shown in several ways that the pressure changes measured with the stomach and duodenum empty were from closed cavities even when the gastric and duodenal lumen communicated with the exterior by the open cannulae 7 to 9 cm. from the recording tips. In a series of experiments, the metal cannulae were covered with "alba stone cement" which adhered firmly to the skin and precluded leakage between the cannulae and the abdominal wall. The continuous mass

of cement also covered the mouth of the cannulae through which the recording tubes passed and thus escape of gut contents through these channels was prevented. The records obtained using these precautions were identical in all respects with those obtained when the cannulae were simply plugged with vaselined cotton and therefore the cannulae were sealed by the latter method in the majority of the experiments.

Rubber tubes having a 4 mm. bore were introduced into the gut; one tube 4 cm. orad from the antral pressure recorder, the second 4 cm. aborad to the bulbar recorder. The opposite end of both tubes extended to the exterior. The records were not modified by opening or closing these tubes. However, when the tubes to the exterior were fastened by the side of the antral recording tip or the tubes were placed in tandem so the open ends were 3 mm. apart, the antral pressure sharply fell to zero when the tubes to the exterior were opened.

When phasic pressures were taken simultaneously from two recording tips, one in the prepyloric region of a fasting dog and the other 1.5 cm. proximal to it, the records evidently were from different cavities. The proximal curve began and terminated earlier and the peak usually was slightly lower than from the distal cavity. On the other hand, two records made from the same cavity (open tubes 2.5 cm. apart in either end of the perforated metal capsule without a balloon) were identical. If the antrum contains water the tendency to form completely separate cavities decreases and records made from two portions of the antrum show more resemblance to each other.

Basal and phasic pressures. The basal pressures within the antrum and bulb are partly extrinsic, partly intrinsic in origin. As in an inanimate object, the extrinsic portion is related primarily to the intra-abdominal pressure in the vicinity of the structure under observation. With the animal at rest, the intra-abdominal pressure will depend on the postural contractions of the abdominal muscles and the diaphragm, but since the abdomen resembles an inverted glass of water, the pressure is lowest at the top of the cavity and increases progressively at lower levels. The extrinsic pressure also is modified by the weight of organs lying on the structure studied, the traction of tissue dependent from it and the weight of the upper wall of the structure. The intrinsic pressure results from the tonic contraction of the antrum and bulb respectively. The gut exhibits basal pressure when no contractions are in the vicinity of the recording tip, for repeated fluoroscopic observations of animals given barium sulfate either with a meal or as an "outliner" showed the region to be quiescent while displaying only basal pressure.

With the animal lying in the dorsal position, basal antral and bulbar pressures during periods of fasting vary between -3 and $+3$ cm. of water. Superimposed on these are pressure elevations of about 2 cm. with each

inspiration and additional small oscillations due to the pulse are observed. Sub-atmospheric pressures, occasionally reaching −8 cm., are most frequent and most marked in the bulb. Usually negative pressures persist for about 1 to 2 seconds but they may continue for 8 to 10 seconds. The pressure for 10 to 15 minutes is sometimes sub-atmospheric as much of the time as it exceeds atmospheric. Transient negative pressure waves are especially frequent immediately after a phasic wave. They are rarely obtained immediately preceding a phasic wave when they might be antici- pated if a wave of relaxation precedes a wave of contraction as implied by Bayliss and Starling's Law of the Intestine. The enlargement of the gut which precedes a peristaltic wave is ascribed by Alvarez (3) and Henderson (4) to the distending pressure of advancing gut contents. Our results fail to support this suggestion for an elevation of pressure rarely precedes a phasic wave in the regions we studied. Wilson and Irving (5) reported negative pressures in the fundus of the full or empty stomach, but they employed the balloon method.

If intra-abdominal pressure in the sphincter region is sub-atmospheric, a completely relaxed antrum and bulb should also show negative pres- sures, while higher basal pressures would result from various degrees of antral or bulbar tonus. In preliminary studies using optical registration, we have repeatedly recorded sub-atmospheric intra-abdominal pressures. Negative intra-abdominal pressures have also been reported by Keppich (6), Reprev (7), Wagoner (8), Overholt (9), Lam (10) and others. How- ever, sub-atmospheric pressures of the gut may not result exclusively from negative intra-abdominal pressures.

Basal antral pressure during fasting usually exceeds bulbar pressure by 1 to 2 cm.; thus factors interfering with the free passage of material between the two cavities must be present. This conclusion probably does not require active contraction of the pyloric sphincter, for the anatomical conformation of the sphincter region, its rough surface and mucous plugs may provide sufficient resistance. A higher basal pressure in the antrum than in the bulb may indicate a greater state of tonic activity in the former region.

Phasic augmentation of pressure superimposed on the basal pressure occurs during fasting both in the antrum and bulb approximately 3 to 4 times a minute. Typically, the antral wave begins slightly before the bulbar wave; they reach a maximum simultaneously and the bulb regains the basal level in advance of the antrum. Although deviations from this pattern are sometimes encountered, the two waves are so closely related and simultaneous fluoroscopic observations show antral, sphincter and bulbar contractions in such regular sequence as to preclude a chance rela- tion. A passage of influences from antrum to bulb is strongly indicated. This observation is in accord with the demonstration by Meschan and

Quigley of the progressive character of the contraction process in the antrum, sphincter and bulb. On the contrary, Joseph and Meltzer (11) report that after each antral contraction the duodenum stops its rhythmic contraction and loses its tone, a manifestation of the Law of Contrary Innervation. Alvarez and Mahoney (12) emphasize a blocking of peristaltic waves at the sphincter; Thomas and Crider (13) find the gastric influence on the duodenum is predominantly inhibitory and Barclay (14) reports that antral peristalsis stops dead at the sphincter and does not pass into the duodenum. The influence transmitted from the antrum to the bulb to produce the sequential relationship noted by us may be either nervous or mechanical. It need not be dependent on the filling of the bulb by antral contents for the relation holds during fasting when nothing is being evacuated from the stomach.

Antral phasic pressure waves persist for 5 to 7 seconds, bulbar waves for 2 to 4 seconds. Maximal pressures are essentially the same in the antrum and bulb and usually range between 15 and 30 cm., but occasionally pressures of 60 to 90 are obtained. Employing the water manometer or tambour technic von Pfüngen (15) recorded a pressure of 162 cm. in the prepyloric region, Moritz (16) observed cyclic prepyloric pressures of 20 to 30 cm., or occasionally 40 to 60 cm., while Sick (17) obtained pressures of 25 to 42 cm. near the pylorus. In fasting animals we find periods of phasic pressure changes continuing for 5 to 10 minutes alternate with periods of quiescence lasting for approximately 60 minutes. The periodic character resembles ordinary hunger contractions recorded from the body of the stomach rather than the continuous activity recorded by Meschan and Quigley (2) from the sphincter region. Alvarez (3), Barclay (14), Wilson and Irving (5), and Goette and Grosser (18) state that roentgen cinematographic and roentgenographic or balloon studies on man and animals show that peristaltic contractions never close the antrum from the orad portion of the stomach and therefore peristalsis is incapable of elevating pressure in the antral lumen. Our results show this description is generally applicable to the proximal antrum but when the wave involves the prepyloric region, a closed cavity develops and the pressure rises. Also, reference to the formula $P = \frac{dv}{dt} R$ shows that if the contraction is rapid, so the rate of volume change is high, pressure may develop even though the lumen is not completely occluded by the wave.

Fluoroscopic studies in our animals when given $BaSO_4$ show that antral and bulbar phasic pressure changes failed to occur without associated contractions. We conclude that antral phasic waves develop when a contraction wave decreases the volume of the cavity occupied by the recording tip and interferes with orad escape of contents at a time when sphincter closure or bulbar resistance obstructs aborad passage of material. In the bulb, phasic pressure develops when the bulb contracts while the

sphincter, orad and the duodenal tone aborad offer resistance to the movement of bulbar contents. A further indication that the sphincter closes and separates the two cavities at the time phasic pressures develop arises from the observation that antral and bulbar pressure changes are rarely identical in magnitude or in time of onset and termination. However, when the pyloric sphincter of fasting animals is propped open with a spool-shaped object having a 5 mm. bore, thus providing free communication between the two cavities, basal pressures in the antrum and bulb agree and phasic pressures coincide in all respects.

Caution must be exercised in expressing phasic pressure changes in terms of peristaltic contractions, for, although they are related, the relationship is not simple. The slope of a pressure curve does not necessarily indicate the rate of gut contraction or relaxation, e.g., a slight relaxation may suffice to connect a cavity of high pressure with one of low pressure, resulting in a rapid fall of pressure in the former cavity, although the gut muscle continues to relax long after the intralumen pressure approaches the basal level.

Ingestion of food. When 150 cc. of thin, strained mush (cornmeal and meat) is administered orally while a series of phasic antral and bulbar pressure variations is in progress, they are replaced by irregular fluctuations of 0.5 cm. of water magnitude occurring 30 to 50 times per minute. Fluoroscopic observations show that peristaltic contractions are inhibited. At the cessation of deglutition the normal antral and bulbar pressure pattern and the peristaltic contractions are promptly regained. If phasic pressure changes and peristaltic contractions are absent preceding the ingestion of food, they usually appear shortly afterwards. If food is simply placed near the animal's nose, phasic changes also disappear but reappear within two minutes following removal of the olfactory stimulus. This influence from eating or smelling food is equally effective in the full or empty stomach. It occurs in vagotomized animals and thus the vagi need not be involved in the reflex.

If the mush is introduced *via* the gastric cannula without attracting the dog's attention the phasic changes do not disappear but, on the contrary, are usually exaggerated. This occurs despite the fact that the rate of introduction is only $\frac{1}{2}$ to $\frac{1}{3}$ as rapid as when the meal is fed by mouth. Whether the food is given orally or by cannula, within a few minutes after feeding, the gradient of pressure from antrum to bulb increases, the phasic changes become more frequent, more uniform and usually of greater magnitude, and this type of activity persists for an hour or more. The records closely resemble those obtained from the pyloric region of fasting or fed dogs by the tandem balloon technic of Meschan and Quigley (2) and tend to confirm the impression that even in the fasting dog the tandem balloon, perhaps because of its distending action, records the "fed" type of motility.

When food is administered orally or by cannula, the basal pressures are

elevated and the gradient of pressure from antrum to bulb is increased. Subatmospheric pressures are still obtained occasionally, especially following a phasic wave. Cannula feeding usually produces an immediate rise in basal pressure but with oral feeding it may be delayed for a few minutes. After 150 cc. of mush, antral pressure reaches 4 to 6 cm., bulbar pressure 1 to 3 cm., but with 1 liter of mush, antral pressure may be 7 to 8 cm. and bulbar about 4 cm. Identical results were obtained in double vagotomized animals. The elevation of antral and bulbar basal pressure after feeding may be due either to a general increase in the tone of the antrum and bulb or to an increase in the quantity of material entering these cavities. Against the latter suggestion is the fact that the typical increase occurs even on those occasions when not enough of the meal enters the antrum and bulb for some minutes after feeding to permit their visualization. Subsequently, the quantity entering the antrum at one time is moderate. The delayed rise in pressure after oral feeding and the immediate response to cannula feeding must involve a reflex producing a difference in tonus development.

Employing dogs several months after double vagotomy we obtained pressure records indistinguishable as regards basal pressure, frequency and magnitude of phasic changes from those of normal dogs except the former showed a slower onset of phasic changes following the ingestion of food. The literature describing the gastric effects of vagus section is contradictory, but these observations are in accord with the report of McCrea, McSwiney and Stoppford (19) of normal gastric motility after vagotomy. However, they do not support the observation of Meek and Herrin (20) who reported a permanent loss of gastric tonus following this operation.

We are grateful for technical aid in this investigation from Miss M. R. Read and Mr. F. J. Rack.

SUMMARY

A critical analysis of the ordinary balloon-water manometer method emphasizes its inadequacy for the registration of gastro-intestinal pressures. Many objections are related to the high volume-pressure ratio, but in addition the recorded pressures will vary with the size, shape and volume of the balloon. Pressures are accurately measured from an open tip in the gut and an optical manometer for registration. The basal pressure of the pyloric antrum usually exceeds the basal pressure in the duodenal bulb and both rise moderately when food enters the stomach. Sub-atmospheric pressures are common in both regions. Periodically, phasic pressure changes amounting to about 30 cm. of water develop in both regions. The phasic pressure changes of the bulb are generally closely related to those in the antrum. They are produced respectively by bulbar or antral contractions. Swallowing or smelling food produces a transient inhibition

of these pressure changes even in vagotomized animals. After feeding, this inhibition is quickly supplanted by phasic changes more uniform, more persistent and frequently of greater magnitude than preceding the feeding.

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